THE OHIO JOURNAL OF SCIENCE

VOL. XLV MAY, 1945 No. 3

EPIPHYTOLOGY OF WINTER WHEAT MOSAIC

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In the spring of 1919 there appeared in Madison County, Illinois, a disease in winter wheat previously unknown in the United States. Subsequent observations revealed the same disease existing in Indiana. The disease has been referred to by such names as "take-all," "so-called-take-all," "wheat rosette," and finally "wheat mosaic," after the causal agent was proved to be a virus. Previous to the discovery of the virus nature of the malady by McKinney (17), speculation arose that the trouble was caused by the feeding of certain insects, such as the Hession fly, *Phytophaga destructor* Say, wheat straw worm *Hormolita grandis* Riley, or wheat stem maggot *Miromyza americana* Fitch, or that unfavorable soil relations were responsible (22). Wheat mosaic virus *Marmor tritici* H.[2] is of unusual interest because under natural conditions the causal agent is transmitted through the soil (16, 17, 30). When seed of a susceptible variety of winter wheat is planted in virus-infested soil in the autumn, disease symptoms do not usually appear in the plants until the following spring after winter dormancy is broken (16, 32). Furthermore, soil which previously has produced a diseased crop of wheat will retain the virus in an active state for at least six years even though no susceptible plants are present during this interval.

Because of these interesting facts the writer undertook a study of the disease to determine, if possible, how the plants became inoculated under natural field conditions. It is the purpose of the present paper to discuss some experiments on this and related problems.

GEOGRAPHIC DISTRIBUTION

In the United States wheat mosaic has now been reported from Illinois, Indiana, Kansas, Maryland, Nebraska, North Carolina and Virginia (20). Wheat mosaic has been found also in Egypt (14), Japan (29) and Russia, where it is present in almost all provinces where winter wheat is grown (33).

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[2]The Latin name of the virus follows the system of nomenclature in the Handbook of Phytopathogenic Viruses (4).

Symptomatology

It is not possible in this paper to give the characteristics of each virus known to cause mosaic in wheat, but only a brief description of the disease will be given as the symptoms appear on susceptible varieties infected under field conditions in the area east of the Mississippi river. Very detailed and comprehensive descriptions of disease symptoms have been made by other investigators (6, 16, 20, 22, 23).

Field symptoms

Wheat mosaic is best recognized in the spring. The field is spotted with areas containing diseased plants scattered at random regardless of the soil type or condition. The plants in these areas are severely dwarfed and in some instances may be dead and lying on the surface of the ground with a few healthy appearing plants present among the diseased, thus giving an uneven appearance. The affected areas are irregular in shape, varying in size from a few feet in circumference to patches which may comprise almost the entire field. The margins of these patches are more sharply defined than those circumscribing diseased areas caused by unfavorable soil relations.

Host symptoms

There are two types of symptoms of wheat mosaic, depending upon the variety of wheat observed. In the variety Harvest Queen, a rosetted condition develops which is characterized by excessive tillering, giving the plants an unusually dwarfed, compact appearance. Such plants are darker green than healthy ones.

In very susceptible varieties such as Purdue No. 1, Purkof and Illinois No. 2, wheat mosaic virus produces stunting without excessive tillering in addition to a mottled condition consisting of light yellow areas intermingled with the normal green (Fig. 1). These light yellow patches may be nearly circular to oblong in shape, or may take the form of large chlorotic streaks which are parallel to the leaf veins. Similar characteristics are seen on the glumes, leaves, leaf-sheaths and stems. This type of mottling is generally referred to as yellow mosaic in contrast with green mosaic which exhibits a mottle consisting of small patches or streaks of a darker green color than normally present in healthy plants. Diseased plants may survive the acute phase of the disease and produce imperfectly filled spikes which are shorter than the spikes of healthy plants.

Histopathology

Microscopic examinations of stained sections of diseased plants from areas where the virus is carried in the soil reveal intracellular vacuolated bodies in the host cells (21). Similar bodies are not found in healthy plants and this fact has been used to determine whether plants are infected if no other macroscopic symptoms are visible. This criterion is not reliable in all cases, since no intracellular bodies are produced in wheat infected with the virus (18) occurring west of the Mississippi river. These vacuolated bodies may occur singly or in groups of two or three, and may be found in any position within the cytoplasm. Similar bodies have also been described from mosaic diseased wheat in Japan (28, 29) and Russia (35).

Experiments on Virus Transmission

Mechanical methods

Wheat mosaic virus is transmitted with difficulty by mechanical means from diseased to healthy susceptible wheat. It has been shown that cool temperatures are favorable for infection (20, 30), but since these conditions do not always exist in most greenhouses in the spring and summer, considerable difficulty is encountered in keeping a viable culture of the virus throughout the year. This is

especially true when one considers that the greatest source of the virus is lost after field-infected plants are matured.

In an attempt to determine the best method of inoculation, the rubbing method described by Jones (8) was compared with the needle-prick method. Carborundum powder was dusted over the plants before inoculation with infectious plant juice which was prepared by grinding parts of diseased plants in a mortar with a pestle. The mortar and pestle were previously sterilized, and a few drops of tap water were added to the inoculum to facilitate inoculation. Only young plants in a stage of rapid growth were inoculated. For the needle-prick method of inoculation a small piece of cotton was wrapped around the point of a dissecting needle allowing the point to protrude slightly through the cotton. The needle-point was kept moist by dipping the cotton in the inoculum at frequent intervals, after which the plants were pricked in several places. Inoculations were made mostly on the leaves and stems but in one experiment plants were inoculated on the roots. In this case the plants were first grown in sand then removed and the debris washed from the roots in running tap water, after which they were inoculated and transplanted in non-infested soil in clay pots. Infectious plant juice was extracted from either the roots or tops of plants and used separately as inoculum in comparative tests.

Table I summarizes the results obtained with four susceptible wheat varieties. It will be noticed from the table that there was no advantage in inoculating the roots, and no infection was obtained with

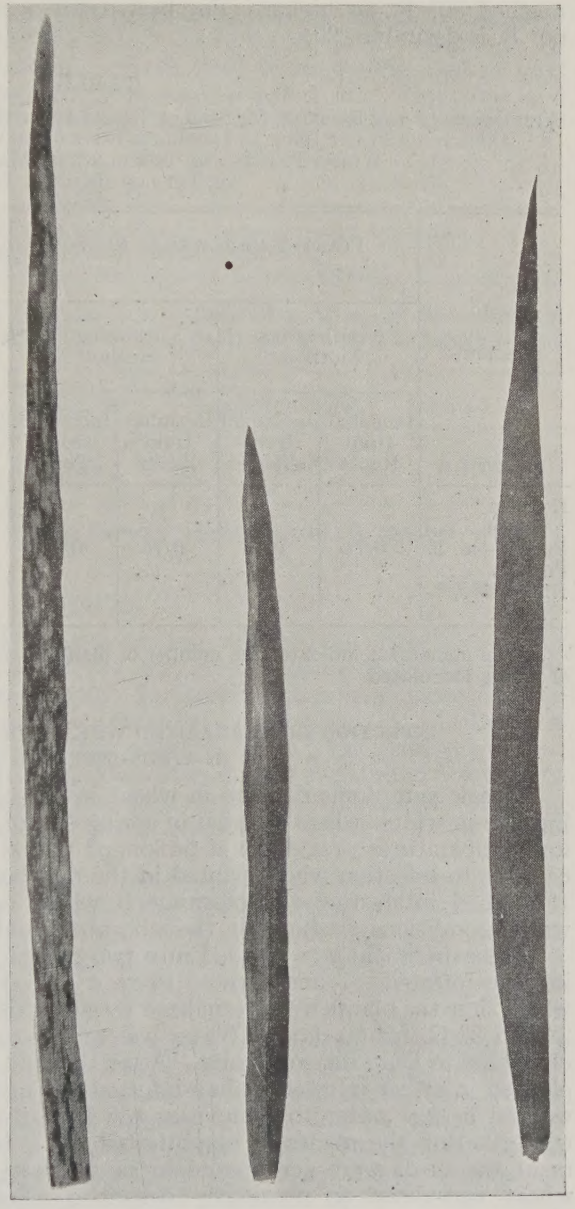

FIG. 1. Symptoms of mosaic in Purdue No. 1 wheat. The two leaves on the left show different types of mottle and are from plants which became infected with virus through the soil. The leaf on the right is healthy.

inoculum extracted from the roots of diseased plants. Neither method of inoculation was very efficient in transmitting the disease; however, these experiments were performed in late spring when the temperature was relatively high and often reached 80° F., or higher. For best results a cool uniform temperature of about 60° F. is desirable (20).

TABLE I

COMPARISON OF THE RUBBING METHOD OF INOCULATION WITH THE NEEDLE-PRICK METHOD, USING INFECTIOUS PLANT JUICE AS INOCULUM EXTRACTED FROM ROOTS OR TOPS OF DISEASED WHEAT PLANTS AND INOCULATIONS MADE IN EITHER ROOTS OR TOPS OF HEALTHY PLANTS

Suscepts	Plants Inoculated in Roots				Plants Inoculated in Leaves and Stems			
	Needle-prick Method		Rubbing Method		Needle-prick Method		Rubbing Method	
	Inoculum from Roots	Inoculum from Tops	Inoculum from Roots	Inoculum from Tops	Inoculum from Roots	Inoculum from Tops	Inoculum from Roots	Inoculum from Tops
Harvest Queen					0/63	0/62	0/38	0/38
Purdue No. 1	0/75[a]	0/75	0/75	0/75	0/73	2/56	0/47	5/43
Purkof					0/59	7/76	0/51	3/32
Illinois No. 2					0/78	3/84	0/67	4/54

[a]The numerator indicates the number of plants diseased and the denominator the number of plants inoculated.

INFECTION OF VERNALIZED WHEAT FOLLOWING GERMINATION IN VIRUS-INFESTED SOIL

Mosaic symptoms develop in wheat in the spring following winter dormancy, but not in winter wheat planted in spring except in rare instances when unusually low temperatures prevail for a prolonged period (20). Spring wheat is also susceptible to infection when planted in the fall, but not when spring sown (18, 20). It seemed interesting to determine if wheat could be infected under artificial winter conditions produced in the laboratory and greenhouse.

The experiment was divided into two parts. In one test the seeds were sown in virus-infested soil and allowed to grow at room temperature for 18 days, after which time the plants were vernalized by subjecting them to a temperature between 2° and 3° C. for 60 days. Water was added to the soil in the low temperature chambers to keep the soil moist. After the cold treatment the plants were transplanted in either infested or non-infested soil in the greenhouse. The roots were washed in tap water to remove as much of the infested soil as possible before transplanting the plants in non-infested soil. In the second part of the experiment the seeds were germinated in sand known to be free from virus, and the wheat vernalized as previously described. Following the cold treatment the plants were transplanted in virus-infested soil.

It will be seen by referring to Table II that it is possible to produce mosaic in winter wheat by duplicating in the laboratory some of the natural conditions to which winter wheat is normally exposed. First, by allowing the plants to grow for a brief period in virus-infested soil and second, by subjecting them to a cold treatment, followed by normal growth development. Webb (30) has shown

that the wheat seedlings become infected in the fall before winter dormancy; this condition also seems to hold true under artificial laboratory conditions, although in this case the percentage of plants showing disease is much lower than under natural field conditions. This result was possibly caused by the small quantity of infested soil used in germinating the wheat, prior to the cold treatment. It has not been possible to produce mosaic in vernalized wheat germinated in sand free from virus, followed by transplanting in virus-infested soil. One thousand seedlings of the variety Purdue No. 1 were treated in this manner and not one plant showed mosaic.

TABLE II

DEVELOPMENT OF MOSAIC IN VERNALIZED WHEAT GERMINATED IN INFESTED AND NON-INFESTED SOIL IN THE LABORATORY

	SEED GERMINATED IN INFESTED SOIL AND PLANTS TRANSPLANTED IN:		SEED GERMINATED IN NON-INFESTED SAND AND PLANTS TRANSPLANTED IN:	
SUSCEPT	Non-infested Soil	Infested Soil	Non-infested soil	Infested Soil
Purdue No. 1...	17/100[a]	15/100	0/100	0/1000

[a]Numerator indicates number of plants diseased, denominator the number of plants transplanted.

INSECTS

It is reported that the wheat virus occurring west of the Mississippi river is transmitted by an unidentified aphid (3), but such evidence is lacking for the virus present east of the Mississippi (18). In Russia it has been demonstrated (33) that the leaf hopper *Laevocephalus* (*Deltocephalus*) *striatus* (L.) DeLong, is a vector of the wheat virus occurring in that country, but it is not definitely known if the Russian virus is related to any of the wheat viruses in the United States. In an attempt to gain some information on the natural spread of the disease, it seemed important to study several species of local insects as vectors of the wheat mosaic virus.

Diseased plants used as a source of the virus were naturally infected by planting the seed in the fall in infested soil. The healthy test-plants, to which the insects were transferred after their viruliferous feed, were grown in clay pots and were about four inches tall. Insect cages made of celluloid were placed over the wheat and pushed into the soil to the depth of one inch. Wheat of the variety Purdue No. 1 was used exclusively in these tests.

One hundred twenty-five mature leaf hoppers, *Laevocephalus striatus*, were collected from a pasture and placed under a cage with diseased wheat. There was a high mortality among the insects. Only 23 adults remained alive after a feeding period of seven days when they were transferred to five healthy wheat plants. Seven days later the insects were removed and the plants observed for mosaic symptoms, but no disease developed up to the time when the plants were in bloom.

In another experiment the leaf hopper, *Agalia sanguinolenta* Prov., was tested. Twenty adult insects were fed on diseased plants for six days, then transferred to five healthy wheat plants where they fed for an additional 17 days. No mosaic had developed on the wheat after an observation period of 60 days.

The third experiment consisted of testing the leaf hopper, *Agalia constricta* (Van Duzee) and both macropterous and brachypterous forms of the fulgorid *Delphacodes campestris* (Van Duzee) as vectors of the virus. There were 19

insects of *A. constricta* and 60 of *D. campestris*. All insects were placed in one cage and fed on diseased wheat. After a feeding period of six days the insects were removed and separated according to species. Each group was then transferred to four healthy wheat seedlings in separate cages. Daily transfers were made to a new set of plants from April 30 to May 21, but in no case did any plants become diseased with mosaic.

One of the common aphids feeding on wheat is *Toxicoptera gramineum* Rond. Specimens of this insect were fed on diseased wheat for three days, then transferred and fed an additional six days on healthy wheat. No mosaic developed in the test plants. In a second trial the same species of insects were fed on diseased plants for six days, then transferred to 50 healthy vernalized wheat seedlings with five mature aphids placed on each plant. After six days the insects were destroyed by fumigation and the plants observed for mosaic symptoms, but there was no evidence of virus transmission.

It must be concluded that the insects tested are not vectors of the virus under the conditions of these experiments.

NEMATODES

As will be seen from the following discussion, there is a certain amount of information which suggests that a soil vector of some kind is responsible for transmission of the wheat virus. It is known that the virus is not carried in the water drained from infested soil (31), and if insects hatched from eggs deposited in infested soil were responsible for transmission it could be expected that healthy, susceptible wheat grown in non-infested soil in alternate rows with plants in infested soil would also become inoculated as the insects emerged from the soil. This does not happen, as only the plants in the virus-infested soil show disease. Furthermore, insects emerging from the infested soil could be trapped by inserting an insect-proof cage over the soil. This the writer has attempted to do, but no insects were found in the cages. It has also been reported by McKinney (20) and confirmed by the writer that the disease develops in wheat grown in infested soil in cages which excludes all outside insects. This circumstantial evidence may suggest that the vector is not necessarily an insect, but some other organism present in the soil. Such an organism must be able to resist periods of alternate moisture and drought for a considerable length of time, as infested soil which has been air dried for at least three years produces just as severely infected plants when replanted to wheat as similar soils cultivated regularly (20).

One kind of soil-borne organism which fulfills these requirements is the nematode. It is known that species of nematodes are able to remain dormant from five to ten years (2, 27) and resume parasitism upon the advent of favorable moisture conditions. The feeding technique of plant parasitic nematodes has been studied in considerable detail by Linford (11, 12, 13). From this work it is concluded that the nematodes puncture the host cells with their stylets and injest the cell contents into their bodies by the pulsation of their esophageal bulbs. There is also evidence that saliva is injected through the stylet of the parasite into the host (11). This method of feeding is similar to that of certain insects which are important vectors of many plant viruses. From these considerations it seemed worth while to study the nematodes normally present in virus-infested soil with regard to their capacity to act as vectors of the wheat virus.

The nematodes were separated from the virus-infested soil by employing the well-known Baermann technique as described by Cort et al., (1). The apparatus used in this study is shown in Fig. 2. The nematodes were collected in watch glasses by opening the pinch cock on the funnel from six to twelve hours after the soil was flooded with water. The nemas were then drawn up in a pipette made from glass tubing, one end of which was drawn out to a fine point and either transferred directly to non-infested soil planted to a susceptible variety of wheat or

transferred to Petri plates containing cultures of the fungus Fusarium. The nemas multiplied rapidly on the fungus isolates and after two weeks time, from the date the nema cultures were started, the substratum was covered with nematodes in all stages of development from eggs to mature individuals. The nemas

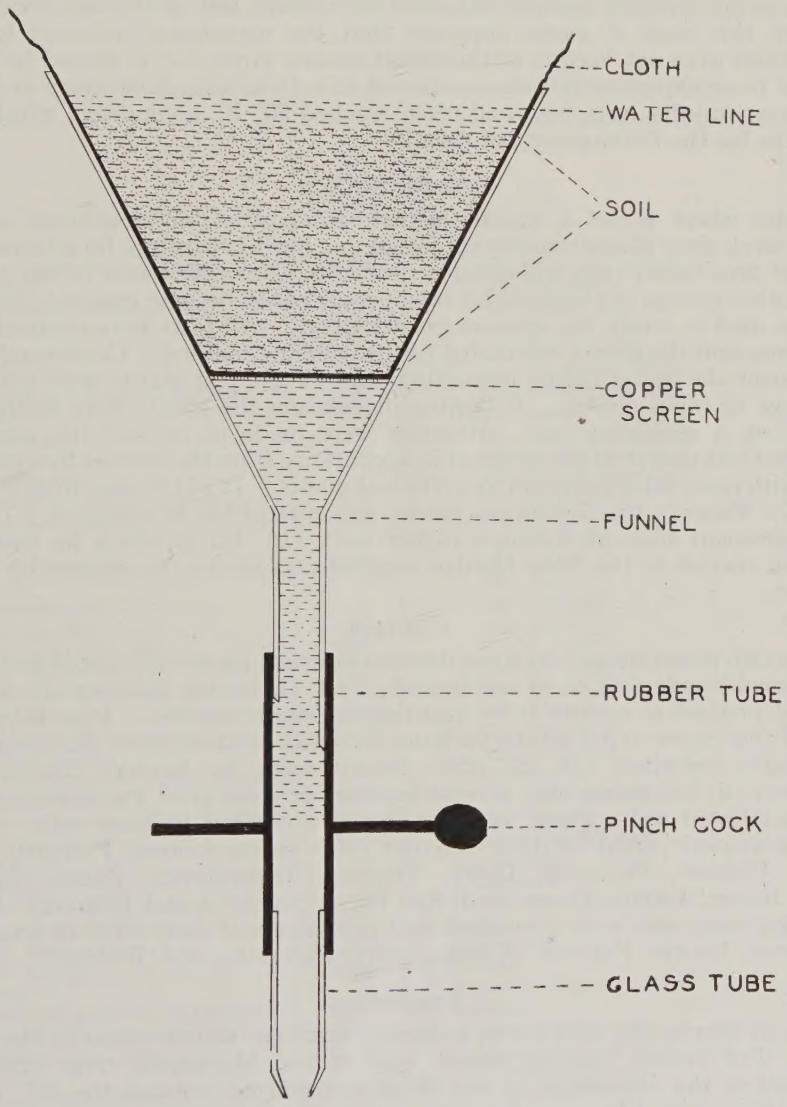

FIG. 2. Diagram of apparatus used to separate nematodes from soil.

were separated from the agar by inverting the Petri plates in water. After four hours the nemas were drained off in great abundance and transferred to non-infested soil planted to wheat. In some cases vernalized wheat was used to test for virus transmission. These trials have been repeated six times and many hundreds of plants were involved, but in no case was there evidence that the nematodes transmitted the virus.

In subsequent tests wheat seeds were surface sterilized and germinated in a water-agar medium in Petri plates to which nematodes previously separated from virus-infested soil were added. The nemas multiplied rapidly and were seen in great abundance on the roots of the wheat; however, it could not definitely be ascertained if the nemas actually fed on the roots. The plants were later transplanted in non-infested soil and observed for mosaic, but no disease developed.

From this work it seems apparent that the nematodes collected from the infested soil were not vectors of the wheat mosaic virus, but it should be pointed out that these experiments were conducted at a time when laboratory and greenhouse temperatures were high and often reached 90° F., a condition which is not conducive for the development of mosaic.

Suscept Range

Winter wheat is not a suitable experimental plant for greenhouse and laboratory work since plants must be subjected to a low temperature for a considerable period of time before they will develop normally. For this reason it was desirable to test other species of plants which might more easily become infected, and which could be used to study the virus in greater detail. All tests were conducted in a greenhouse and the plants inoculated by the rubbing method. Carborundum was dusted over the leaves before inoculation and all the test plants were young and in a stage of rapid growth. Following inoculation the plants were washed with water from a sprinkling can. Attempts were made to recover the virus from inoculated test plants which deviated in appearance from the normal by inoculating wheat with plant juice extracted from the test plants. It will be seen from Table III that only wheat in the Gramineae family was susceptible to infection. This fact is in agreement with McKinney's earlier work (16, 18) in which he found that all cereal species in the tribe Hordae were susceptible to the disease by natural infection.

Control

Although wheat mosaic does not develop in wheat grown in infested soil treated with formaldehyde (16) or in soil heated at 60° C. for ten minutes (7), the only practical method of control is by planting resistant varieties. It is beyond the scope of this paper to list all the varieties reported resistant since this information is available elsewhere (16, 23, 32). Recent work by Koehler, Bonnett, and McKinney (9) has shown that several varieties produce good yields when planted in virus-infested soil. These varieties, listed according to their order of yield over a four-year period in three different fields are as follows: Fulhard, Prairie, Nabob, Wabash, Fulcaster, Duffy, Thorne, Cooperatorka, Fulhio, Michigan Amber, Inivira, Harvest Queen 34–1, Red Wave, Shepherd, and Trumbull. Records on lodging resistance were also taken and in this regard their order of importance is: Thorne, Prairie, Fulhard, Nabob, Duffy, Fulcaster, and Wabash.

Discussion

Several viruses are reported as inducing mosaic in winter wheat in the United States. The viruses causing mosaic west of the Mississippi river differ from those east of the Mississippi in not being transmitted through the soil, and are reported transmitted by an aphid, while no insect-vector is known to transmit the eastern viruses which are carried with the soil. The two viruses may also be differentiated upon the fact that vacuolated bodies are found in the cells of plants infected with the eastern virus, while no such inclusions are produced by the western virus (18).

Two types of mosaic are described from the west (19), namely, green mosaic and yellow mosaic. The latter appears to cause the most pronounced damage, and more recently McKinney (20) isolated seven different viruses from diseased

plants grown east and west of the Mississippi river. The distinctive characteristics were based on the symptoms produced in Harvest Queen wheat grown under a definite photoperiod, host range, and whether the viruses were transmitted through the soil. Each wheat virus was designated by a numeral from one through seven. It appears to the writer that a better method would be to consider only two distinct viruses as causing wheat mosaic in the United States. Wheat mosaic

TABLE III

PLANTS TESTED FOR SUSCEPTIBILITY TO THE WHEAT MOSAIC VIRUS

	No. Plants Tested[a]
Chenopodiaceae	
**Beta vulgaris* L. (sugar beet)[b]	0/10
Compositae	
Callistephas chinensis Nees	0/10
**Helianthus annus* L.	0/25
Lactuca sativa L.	0/7
Fagetes erecta L.	0/6
Taraxacum officinalie Weber	0/5
**Zinnia elegans* Jacq.	0/20
Cucurbitaceae	
Cucumis melo L.	0/7
C. sativus L.	
var. Evergreen Pickling	0/32
var. Longfellow	0/29
Cruciferae	
Brassica rapa L.	0/20
Gramineae	
Oryza sativa L.	
var. Acadia	0/26
var. Blue Rose 41	0/24
var. Early Prolific	0/29
var. Improved Blue Rose	0/29
var. Rexoro	0/20
Saccharum officinarum L.	0/10
Sorghum vulgare	
var. caffrorum (Thunb.) Hubb. & Rehder	0/30
var. *saccharatum* (L.) Boerb.	
*Amber	0/38
*Atlas	0/48
Triticum aestivum L.	
var. Harvest Queen	0/76
var. Illinois No. 1	3/84
var. Purdue No. 1	5/68
var. Purkof	3/83
Zea mays L.	
*Hybrid Golden Cross Bantam	0/50
*Hybrid Indiana 616	0/50
*Hybrid Iowa 939	0/50
Leguminosae	
Medicago lupulina L.	0/10
Phaseolus aureus Roxb.	
*var. Natives (so-called)	0/50
*var. Purdue	0/50
*var. Select, No. 12	0/50
P. vulgaris L.	
*var. Early Golden Cluster	0/50
var. Kentucky Wonder	0/14
var. Unrivalled Wax	0/16
Pisum sativum L.	
var. Laxtons Progress	0/25
var. Little Marvel	0/25
var. Notts Excelsior	0/25
var. Potlatch	0/25
Soya max (L.) Piper	
var. Canaday	0/12
var. Chief	0/12
var. D-1	0/14
var. Dunfield	0/15
var. Illini	0/15
var. L 6-12	0/12
var. L 6-685	0/16
var. Mandarin	0/14
var. Mingo	0/13
var. Patoka	0/14
var. Richland	0/14
var. Scioto	0/15
var. Wisconsin 606	0/14
var. Wynco	0/10
Trifolium incarnatum L.	0/10
T. pratense L.	0/10
T. repens L.	0/10
**Vigna sinensis* (L.) Endb.	0/50
Liliaceae	
Allium cepa L.	0/5
Linaceae	
Linum usitatissimum L.	0/12
Solanaceae	
Capsicum frutescens L.	0/3
Lycopersicon esculentum Mill.	0/10
Nicotiana glutinosa L.	0/10
N. rustica L.	0/10
N. tabacum L.	0/10
Solanum nigrum L.	0/7

[a]The numerator indicates the number of plants diseased; the denominator indicates the number of plants inoculated.

[b]Plants checked with an asterisk indicates these plants showed unusual symptoms following inoculation and that attempts were made to recover virus from them by using wheat as a test plant; but no virus could be demonstrated.

virus occurring east of the Mississippi river and classified by Holmes (4) as *Marmor tritici* H., would retain the name wheat virus 1, according to the numerical classification. What McKinney refers to as wheat virus 2 and wheat virus 3 may be regarded as strains of wheat virus 1, and thus would be designated as wheat virus 1A and 1B, respectively. The viruses occurring west of the Mississippi may be designated as wheat virus 2, with the virus referred to by McKinney as wheat virus 4 considered the type strain. Following this system further, wheat viruses 5, 6 and 7 would be regarded as wheat viruses 2A, 2B, and 2C, respectively. Until more is known about the distinctive properties, a Latin binomial is not suggested for the western virus.

The viruses inducing mosaic of wheat in Japan are probably closely related to the eastern virus in the United States (5, 28). Two types of mosaic have been described, namely, green mosaic and yellow mosaic. These are distinguished by the difference in vacuolated bodies present in the host cells of diseased plants and by differential wheat varieties susceptible to the viruses (29, 15).

In Russia there appears to be two distinct viruses capable of causing mosaic in cereals, the virus causing mosaic of oats known as "zakooklivanie" (pupation disease), and the virus of winter wheat mosaic. Both viruses are infectious for both oats and wheat as well as other plants, but certain characteristics differentiate them. The virus causing "zakooklivanie" produces vacuolated bodies and protein crystals in cells of infected plants, while the wheat virus does not induce the formation of protein crystals (24). In diseased spring crops the oat virus causes excessive tillering, while the wheat virus seems to have no such effect (34). Furthermore, different insect vectors are involved. The virus causing mosaic in oats, "zakooklivanie," is transmitted from diseased to healthy plants by *Delphax striatella* Fallen (26), while *Laevocephalus* (*Deltocephalus*) *striatus* (L.) De Long, is reported a vector of the wheat mosaic virus (33). Neither virus is transmitted by mechanical methods and wheat does not become infected through the soil (34), although the reports on infection through the soil by the oat virus are in conflict (10, 25).

What the relationship is between the Russian viruses and those present in the United States is not clear. If we compare soil transmission then the wheat virus in Russia is much like our western virus, and if we consider the histopathology then the Russian virus compares favorably with our eastern virus since both induce the formation of intracellular inclusions.

There is not enough information on the wheat virus occurring in Egypt to make comparisons with any of the other known cereal viruses.

From what is known about the wheat mosaic virus occurring east of the Mississippi river in the United States, the writer is constrained to believe that a soil-borne organism is the vector. This might be an insect, or some other parasite living in the soil. Although nematodes did not transmit the virus in this study, the writer feels that more work should be done on this problem. It is possible that the technique employed was inadequate to demonstrate this point. For instance, the next logical step would be to subject the plants to winter conditions after the nematodes were allowed to come in contact with the wheat plants to be inoculated.

Summary

Wheat mosaic is present in Illinois, Indiana, Kansas, Maryland, Nebraska, North Carolina and Virginia. From abroad the disease has been reported in Egypt, Japan and Russia.

The virus causing the disease east of the Mississippi river is transmitted to susceptible plants through the soil and produces two distinct types of symptoms. In the variety Harvest Queen a stunted, rosetted condition is produced, with or without mottling. In other varieties such as Purdue No. 1, Purkof, and Illinois

No. 2, stunting is pronounced without excessive tillering. In these varieties a mosaic mottling is the predominant early symptom of disease. Vacuolated intracellular bodies are present in cells of diseased plants, but not in healthy.

The virus is transmitted, with difficulty, from diseased to healthy wheat plants by mechanical means. Inoculum extracted from roots of diseased plants did not produce infection in healthy plants by the rubbing method of inoculation nor by the needle-prick method. It is possible to induce the disease in wheat by artificially subjecting the plants to the normal temperatures to which winter wheat is exposed after allowing the plants to grow in virus-infested soil for 18 days.

The insects, *Laevocephalus* (*Deltocephalus*) *striatus*, *Agalia sanguinolenta*, *A. constricta*, *Delphacodes campestris*, and *Toxicoptera gramineum*, did not transmit the virus. Nematodes were also tested as vectors but the results obtained, with regards to transmission, were negative.

Several species of plants were tested for susceptibility to the disease by mechanical inoculation, but symptoms were produced only in wheat.

The only practical method of control in areas where the disease is prevalent is to grow resistant varieties. Several of these have been found to produce good yields on virus-infested land.

Two viruses may be considered as causing mosaic of winter wheat in the United States. The virus east of the Mississippi river is transmitted through the soil and induces the formation of vacuolated intracellular bodies in cells of diseased plants. The western virus is not transmitted through the soil and produces no intracellular bodies in cells of infected plants.

The virus causing mosaic in Japan is probably closely related to the eastern virus present in the United States, but the wheat virus in Russia has characteristics of both our eastern and western viruses.

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THE ALGOLOGIST AND WATER SANITATION[1]

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A potable water is a necessary adjunct to the devlopment and well being of a community. Each member expects, as a part of his communital rights, a palatable and pure water supply. If the water becomes distasteful because of sediment, peculiar flavors and odors, or if drinking it results in illness, then, and only then, will he give any consideration or thought as to the source, contaminating factors or the chemical and physical treatment which contribute to the purity of the supply.

The highly technical engineering principles and problems connected with the location, construction and operation of reservoirs and purification plants are beyond the scope of this paper. Only those that pertain to the elimination of aquatic organisms which may interfere with the delivery of potable water to the consumer will be considered. Likewise, bacteriological examination is a separate study and will be ignored except in a few instances where the intimate relations between bacteria and plankton involve the algologist.

To the causal observer, the work of the algologist in connection with water supplies is something new in the application of microbiology. Instead, it is a science which has been developing for nearly a century, and if I may predict, will become more and more important as the centralization of populations and industrialization increases in this or any other country.

The industrialization of an area with a subsequent increase in population demands a substantial increase in the water supply. This demand is met by utilizing surface water available in lakes or rivers. Unlike the inadequate, but relatively pure well water, these surface supplies create a major problem in which algae specifically, and microorganisms in general must be combatted.

Although the study of microorganisms began with the invention of the compound microscope near the beginning of the seventeenth century, it was not until about 1850 that their importance in water supplies was recognized. After Hassall in England and Cohn on the continent first directed attention to the importance of microorganisms, the next fifty years saw important and far-reaching investigations carried on by numerous workers in this country as well as in Europe. While these investigations were of basic importance for future work, none of them resulted in a systematic examination of any particular water supply until the Massachusetts State Board of Health in 1887, established and has since maintained such a survey. Numerous cities soon followed its example. However, satisfactory methods to control microorganisms were not known until 1905 when Moore and Kellerman proposed the use of copper sulfate for the elimination of algae. This was the beginning of the modern period of algal control in water supplies.

All natural water sources such as lakes, rivers, streams and ponds, and in some cases, artificial wells which are maintained by underground streams, contain plankton. The name "plankton" is applied in water supply work to all microscopic or near-microscopic organisms, both plant and animal, which maintain a free-floating existence in bodies of open water. Although plant and animal plankton are involved in water sanitation, the microscopic plants or algae, are of

[1]Papers from the Dept. of Botany, The Ohio State University, No. 482. The data pertaining to the Columbus, Ohio, reservoirs were obtained during yearly surveys directed by Mr. Charles P. Hoover, Chief Chemist of the Columbus City Division of Water.

primary importance because they alone can manufacture and contriubte to the food supply of the animal plankton. Elimination of the algae is therefore the first step in the control of the animals.

The identification and the determination of the number of algae present is the initial problem in the treatment of any reservoir. The investigator must be able to identify all common plankton algae, to the species if possible, under relatively low magnification. This is imperative because the counting chambers used do not permit the use of high magnifications. Only trained algologists who are thoroughly familiar with range in forms and structures of the various species encountered can satisfactorily undertake this part of the work.

A great many species of algae may be present in a reservoir but more than likely a few species will be found in sufficient abundance to affect the water supply. Ususally there are great variations in the number of individuals in these species. As all species are not equally obnoxious, quantitative rather than qualitative studies are necessary. It is not the intention here to enumerate all the plankton algae which may be encountered. Such information, if desired, may be readily secured from standard manuals of the algae. The following list does however, present a few of the important flavor and odor producing genera, or which, because of shape and structure may prevent proper filtration and necessitate frequent cleaning or "backwashing" of the filter beds.

BLUE GREEN ALGAE	GREEN ALGAE	YELLOW GREEN ALGAE AND DINOFLAGELLATES	DIATOMS
Anabaena	*Pandorina*	*Synura*	*Asterionella*
Microcystis	*Volvox*	*Uroglena*	*Synedra*
Aphanizomenon	*Tetraspora*	*Dinobryon*	*Fragillaria*
Rivularia	*Spirogyra*	*Ceratium*	*Tabellaria*

The diatom genera *Synedra*, *Fragillaria*, and *Asterionella* cause much of the filtration trouble, although mats of *Spirogyra* or other filamentous algae settling on the filters may result in similar difficulties. These, as well as a number of other genera including those listed above may cause unpleasant odors or flavors. Some algae, for example the species of *Synura* which has the odor of cucumbers, may be identified on the basis of odor alone. Other odors or flavors produced by algae may be described as fishy, grassy, moldy, pig pen, and aromatic such as geranium, violet, or nasturtium. Other than *Synura* which is extremely disagreeable, the blue green algae probably account for a large proportion of bad tasting water because of their wide occurrence and the great rapidity with which they grow and multiply. In all instances the odor is presumably due to oils produced within the cells of the alga and liberated into the water upon their death and decomposition. This explains the sharp increase in the odor intensity of water for a few days following the application of an algicide. Results of bacteriological studies during this period show a great increase in the number of bacteria which is inversely correlated with the decrease of the algae. The intensity of the odors are stated in terms of "The Threshold Odor Number," a figure which represents the dilution of the sample that is required to reduce the odor to a point where it is just detectable. An odor free water is used to dilute the sample. With the realization that Synura oil can be recognized in concentrations as low as one part in twenty million parts of water it becomes apparent that the algae must be eliminated at the source rather than at the pumping station.

The value of the data obtained will depend largely upon the careful selection of stations at which periodic collections are made. These stations should include

areas of greatest plankton concentration as well as those that will yield data concerning the effect of tributary streams entering the reservoir. The extent to which sewage or industrial contamination, algal growth, and commercial or pleasure navigation may be highly important.

Samples may be obtained by one or more of several methods. If a complete tabulation of all organisms is desired, a known quantity of the water may be centrifuged. Another excellent procedure is that known as the Sedgwick-Rafter Method. By this method the organisms are removed from the sample by means of a layer of fine sand supported on bolting silk, then washed from the sand by a measured quantity of distilled water. The objections to this method are the amount of equipment necessary, the time necessary for filtration, and a possible loss of organisms which stick to the sand. If the results justify the means, and extreme accuracy may be sacrificed, then the net method may be used. In this procedure a known amount of water is passed through a net of silk bolting cloth. These nets come in various size mesh but the No. 20 mesh is relatively satisfactory. Although part or all of the small plankton may pass through the net, most of the objectionable species of algae are retained. If the net is handled in the same manner each time a collection is made, and the same amount of water is passed through at each collection, the final result is a figure that represents the relative abundance of the important species of algae present. The writer has used this method with satisfactory results during the past several summers in plankton surveys for the Columbus City Division of Water, Columbus, Ohio.

The resultant filtrate from the net may be made up to any desired volume for easy and rapid computing. In the Columbus survey, 25 gallons of river water are filtered through the net and the resultant sample made up to 50 cc. with a standard preservative. At this concentration, one cc. of sample is equal to one-half gallon of river water. Preservation of the sample is necessary if they are to be kept for even a few hours. If not preserved, decomposition and the use of the algae as food by the zooplankton will render the sample useless. A word of caution here is advisable. Colonies of *Synura*, *Uroglenopsis* and *Aphanizomenon* disintegrate upon preservation. Samples containing these genera should be worked while still living, or immediately after fixation. The latter is better with motile algae as it is almost impossible to count the living organisms. The population is determined by transferring one cc. of the sample to a standard counting chamber holding one cc. and which is one mm. deep. By using a Whipple disc which has been calibrated to give a field of one sq. mm., the number of each species in one cu. mm. of sample is determined. At least ten such "counts" should be made, and for greater accuracy up to twenty-five should be made. The average of these counts multiplied by one thousand will give the average number of each organim in one cc. of the sample. If this one cc. of sample represents one-half gallon of water then the average number of each organism per gallon of water in the reservoir is easily computed by doubling the number in the sample. If it is desirable to have the final results expressed in terms of the metric system one may work with liters instead of gallons. However, if the work is part of an organized program of a City Water Filtration Department, it is well to keep results in the terms ordinarily used by other workers at the station.

Counts should be made at intervals of not more than one week. If the results show a rapid increase of one or more objectionable species, steps toward their elimination should be taken immediately. If development is allowed to continue, the resultant "Water bloom" will be so intense that treatment will result in extremely disagreeable odors. If the bloom is checked early in its development, it will be less difficult to control, and the after effects are far less evident to the consumer. Control should also be initiated if there is a general and steady increase

of all species because the enormous volume of decomposing organic material will produce some disagreeable odors or flavors.

The most satisfactory method for the control of algae in reservoirs, lakes, ponds or pools is that proposed by Moore and Kellerman in 1905. The control, as previously stated in this paper, is accomplished through the use of copper sulfate. This compound as an algicide is cheap, effective, easily applied, and harmless to human beings in the concentrations found effective. Any objections to its use in drinking water are unfounded. Copper is normally present in the human body and many of our foods contain greater quantities of the metal than is found in the water following treatment. When $CuSO_4$ is applied to the water in a reservoir much of it changes to insoluble compounds so that the water which reaches the consumer has an extremely low concentration of soluble copper compounds.

The following list of algae with the concentrations of $CuSO_4$ effective in their elimination should allay fears in this regard. In each instance the figure following the algal name represents the number of parts of water to one part of $CuSO_4$.

Aphanizomenon	5,000,000	*Pandorina*	100,000
Anabaena circinalis	10,000,000	*Volvox*	4,000,000
Asterionella	8,000,000	*Synura*	3,000,000
Synedra	600,000	*Spirogyra*	25,000,000

The above list represents only a portion of an extended list published by Moore and Kellerman in the U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin No. 76, 1905. These concentrations, based on water of average hardness and having a temperature of 59° F., will vary slightly in water at other temperatures and degrees of hardness. The amount of $CuSO_4$ needed is determined on the basis of the volume of the reservoir and the kind of algae present. It may then be applied by towing a gunny sack of the crystals behind a slow moving motor boat or row boat. The process may be expedited by the installation of a pump and mixing unit in a relatively fast motor boat. Powdered $CuSO_4$ is mixed with water pumped from the reservoir and the resultant solution sprayed from the rear of the boat. The advantage of this method is a more uniform and better application with less waste of material and time.

There is no possible way to satisfactorily predict the intervals between applications. Hardness of the water, temperature, and organic content, as well as the reappearance of resistant forms of algae all combine to make such predictions of little value. Satisfactory results depend upon regular periodic surveys by the algologist. In some reservoirs it will be found that treatment is required only during the early spring and late summer or autumn months, and may be correlated with the usual maxima of diatoms at those periods. In others, the development of blue green algae will necessitate continuous treatment throughout the summer. In the Columbus, Ohio, reservoirs, spring treatment is seldom necessary because of high turbidity.

If this turbidity is continuous or nearly continuous during the summer, treatment is either eliminated or reduced to a minimum. An excellent example of this occurred in the Griggs reservoir at Columbus during the summer of 1943. Figure 1 shows graphically that five maxima, based on total organisms present, occurred between July 1 and November 3.

In the first four instances, as the organisms approached proportions indicating the desirability of applying $CuSO_4$, severe storms occurred over the water shed. The resulting turbidity and consequent decrease in light available to the algae reduced photosynthesis. This in turn sharply curtailed the growth and number of algae. The fifth maximum which occurred about October 13, disappeared shortly because of an extended period of cloudy weather, some precipitation, and a sharp drop in temperature. Such turbidity provides an excellent natural control for

the algae. This method has met with success in ponds and shallow lakes where turbidity can be created by artificial means.

In any algal elimination program there is one precautionary measure of which those in charge must be cognizant. If the body of water is open to fishing, it must be remembered that $CuSO_4$ is toxic to fish as well as to algae. Although little work has been done regarding the tolerance of fish in this respect, it is definitely known that the concentrations of copper which they can withstand vary almost as widely as they do in the algae. Likewise, the tolerance to the copper depends to a great extent upon the carbonates present, the temperature, and the amount of organic material. Some of the more common varieties of fish and the concen-

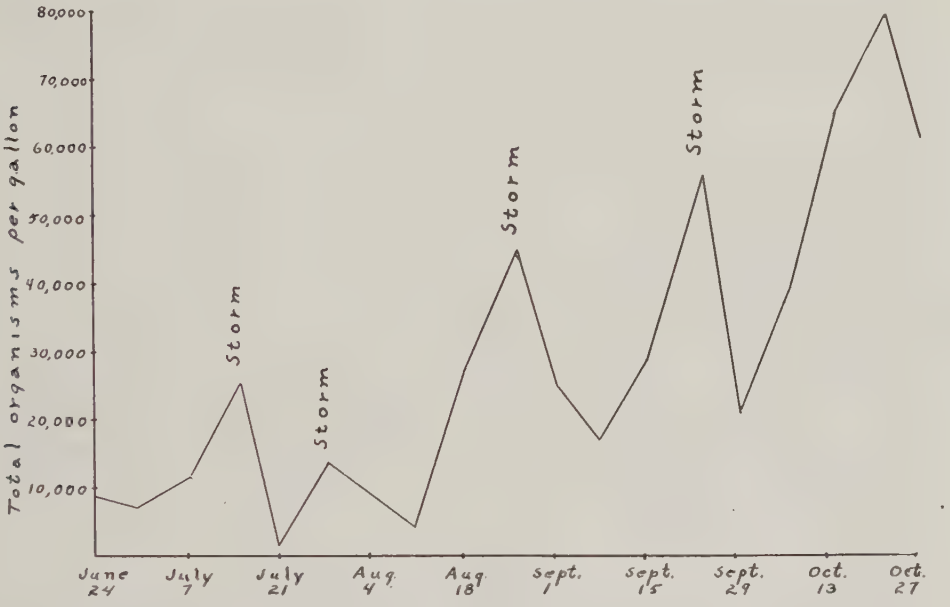

Fig. 1. Graph showing the maxima of total organisms in the Griggs Reservoir of the Columbus City Water Supply, Columbus, Ohio, during the summer of 1943.

trations of $CuSO_4$ which may safely used are given below. These figures are the result of the investigations of Moore and Kellerman. The figures represent the number of parts of water used to one part of $CuSO_4$.

Black Bass	500,000	Catfish	2,500,000
Sunfish	750,000	Carp	3,000,000
Perch	1,500,000	Trout	7,000,000
Goldfish	2,000,000		

Consistent and favorable results during the past forty years prove the efficiency of $CuSO_4$ as an algicide. The tabulation of a few results obtained through its use in the Columbus, Ohio, reservoirs will exemplify this efficiency.

On September 21, 1942, the plankton counts at three stations in the Griggs Reservoir of the Columbus water supply showed a decided increase in the number of organisms present. In order to prevent the development of a late autumn bloom, $CuSO_4$ was applied at the rate of nine pounds per acre. On October 5, counts were again made which showed that the development had been checked

and the number of organisms considerably reduced. During this period weather conditions were relatively constant. A tabulation of the results is given below:

Station No.	Total Organisms per Gal. before Application of $CuSO_4$	Total Organisms per Gal. after Application of $CuSO_4$
1	12,240	1,440
2	9,360	2,400
3	6,480	1,200

Another excellent example of algal control resulted from the investigations of Professor W. L. Huff, who, in 1915, was employed by the city of St. Paul to study the microorganisms of Vadnais Lake, St. Paul, Minnesota. The effects of $CuSO_4$ on the microorganisms are shown graphically in Figure 2.

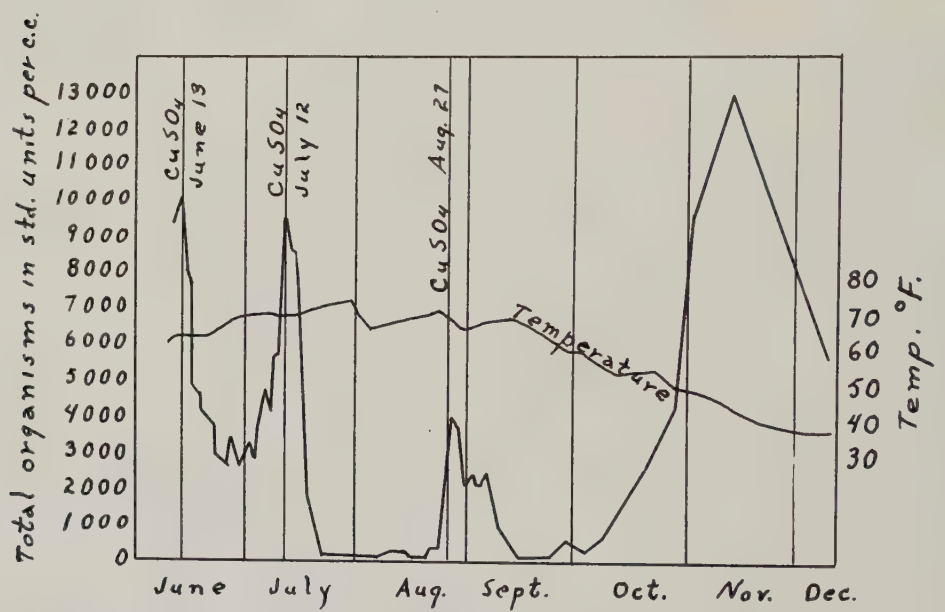

Fig. 2. Results of copper sulfate treatment of Vadnais Lake, St. Paul, Minn., 1915. After Huff.

Results, such as the above are representative and indicate the success of an algal control program.

The intention of the writer throughout the foregoing discussion has been to acquaint the reader with problems connected with algal control in water sanitation. Although most of the methods have been published, it is felt that a summary of the local work will help the average consumer to appreciate the efforts being made to supply him with the best possible water. Also that in the future the problems of water supply control as purity, freedom from odors, flavors, hardness and many other qualities will increase rather than diminish. The solution of these problems will depend upon adequately trained aquatic biologists, bacteriologists and chemists working in co-operation.

ELEVEN NEW LEAFHOPPERS WITH NOTES ON OTHERS (HOMOPTERA: CICADELLIDAE)

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Alconeura socorroana n. sp.

Near *A. planata* Ball and DeLong, but male with processes at apex of aedeagus shaft. Slender, vertex evenly rounded, pronotum one-third longer than vertex, second apical cell pedunculate.

Evenly olive green with pale areas as follows: Anterior margin of vertex and median narrow line; lateral and anterior margins of pronotum, also short antero-median line; scutellum, median line in basal half and lateral line either side defining inner margins of lateral angles; basal area and some irregular spots along claval vein, apical area along veins and two indefinite spots in outer apical cell. Disc of pronotum and inner base of scutellum darker; round black spot in fourth apical cell one-fourth distance from base near vein, and small area at base of cell darkened. Below, fumose but for anterior margin of head.

Inner male genitalia.—Aedeagus in lateral view three times as long as basal width discounting length of pair of apical dorsal retrorse processes which curve back toward shaft opening and are one-third length of shaft: three ventral basal processes parallel with shaft, one median ventral and two lateral, extending a little beyond shaft opening, lateral processes longer. Pygofer process heavy, in dorsal view as long as aedeagus, curved gently inward and tapered to apex.

Female.—Last ventral segment three times as long at middle as broad, narrowed on apical third, produced in a rounded lobe, pygofer narrow with line of six white spines either side of ovipositor, evenly distributed; ovipositor exceeding pygofer.

Length: 2.5 mm.

Male holotype, two male paratypes and allotype, Socorro Co., N. M., July 10, 1940, D. J. & J. N. Knull; three teneral specimens, Tucson, Ariz., May 13, 1941, D. J. & J. N. Knull.

Typhlocyba sciotoensis n. sp.

Resembling *T. luculla* Medler externally and near *T. piscator* McA. in type of inner male genitalia which are very complex.

Chalky white with yellowish tinge on head and pronotum, a darkened scutellum, darker in median basal area, and pronounced dark band over white crossveins, extending farther anteriorly than posteriorly. Eyes dark, vertex with thin impressed median longitudinal line reaching almost to apex, a round spot either side near apex; some subcutaneous darkening of pronotal disc and area adjacent to scutellum. A dark round spot at inner posterior end of M_4, an intensely darkened streak below outer crossvein and another on outer R_3.

Male genitalia.—Outer clasper broad basally, contracted below middle; pygofer truncate on outer margin, exceeded by claspers and aedeagal processes, style with sharp projecting heel and sharply pointed, up-turned toe. Aedeagus in ventral view, a pair of narrow ventral processes arises at base and extends ventrad to shaft for two-thirds its length, arms separated at base by three times width of shaft and parallel entire length. Apex of shaft broadened slightly and curved decidedly ventrad, a pair of incurving processes before apex, a lateral branched pair behind them, and a third longer pair curved sharply ventrad toward apex arises still further back. In lateral view, aedeagus shaft curved narrowing from broad base to complex apex, forming an incomplete semicircle. Shaft somewhat swollen at juncture of processes. Female segment roundly produced; ovipositor dark.

Length: 3 mm.

Male holotype and paratype, Scioto Co., Ohio, June 10 and 17, 1944, D. J. & J. N. Knull, and allotype, Delaware Co., Ohio, July 3, 1942, D. J. & J. N. Knull.

Typhlocyba foliosa n. sp.

Near *T. piscator* McA., from which it differs chiefly in having a much paler dorsum.

Head, pronotum and scutellum ivory, eyes pale, tegmina whitish hyaline, sometimes faintly tinged with yellow; areas between sectors anterior to crossveins, slightly dark clouded, distinct black marks on veins at outer edge of R_1 and on claval margin at apex of vein M_4. Below pale, ovipositor dark and dorsal abdominal segments sometimes narrowly black margined.

Male genitalia.—Similar to those of preceding and also to those of *T. piscator* McA. in general form. In this species, however, aedeagus has ventral basal processes distinctly curved, S-curved in lateral view, and shaft is swollen at base and apex. Two pairs of apical processes, apical shorter, almost complete a circle in lateral view. In ventral view apical processes appear straight and pointed, the others are enlarged leaf-like and curved forward.

Length: 3 mm.

Male holotype and paratypes, four male and one female, Shawnee Forest, Scioto Co., Ohio, June 9, 1943, D. J. & J. N. Knull; allotype and two female paratypes, June 17, 1944; four male paratypes, June 10, 1944; paratypes from other localities: two males, Hocking Co., Ohio, September 16, 1943; one male, Delaware Co., Ohio, June 27, 1943; one female, Lucas Co., Ohio, June 30, 1943; and one male paratype, Great Smoky Mt. National Park, Tenn., June 21, 1942 (this specimen decidedly yellow-tinged), all collected by D. J. & J. N. Knull. Six paratypes in collection of The Ohio State University.

Erythroneura stupkaorum n. sp.

In the maculata group near *E. triangulata* Beamer, but with a distinctly different style in inner male genitalia.

General ground color creamy white, yellowish semihyaline elytra marked with orange. Vertex with three basal pale spots, lateral round and median elongate, surrounded by continuous orange bands; pronotum with median rectangular spot not reaching margins, with translucent anterior lateral arms forming rough Y, spot back of each eye, eyes tan; apex of scutellum orange, remainder pale; clavi with three indistinct spots, not joined, coria with similar spots, one on costa before humeral angle, three around plaque and one near apex of clavus; crossveins faintly reddened, small black spot in base of cell M_4.

Male genitalia.—Pygofer hook single, distinctly S-curved, exceeding pygofer by one-half its length, noticeably thickened on middle half, inner edge of thickened portion serrate. Style with large foot, heel scarcely projecting, almost a right angle, posterior point longer than base of foot, slender, curved very slightly out, sharp. Aedeagus short, heavy, bluntly square, the knob-like apex with lateral wing-like projection, in lateral view narrow, roughened, straight.

Length: 3.25 mm.

Male holotype, allotype and paratypes, Smoky Mt. National Park, Tenn., collected in June by D. J. & J. N. Knull. Three paratypes in collection of The Ohio State University.

Named for Mr. Arthur Stupka, Park Naturalist, and his family.

Erythroneura trautmanae n. sp.

In the maculata group, resembling *E. knighti* Beamer, but with unique pygofer hook.

Creamy white on anterior parts and below, elytra yellowish semihyaline, with orange and

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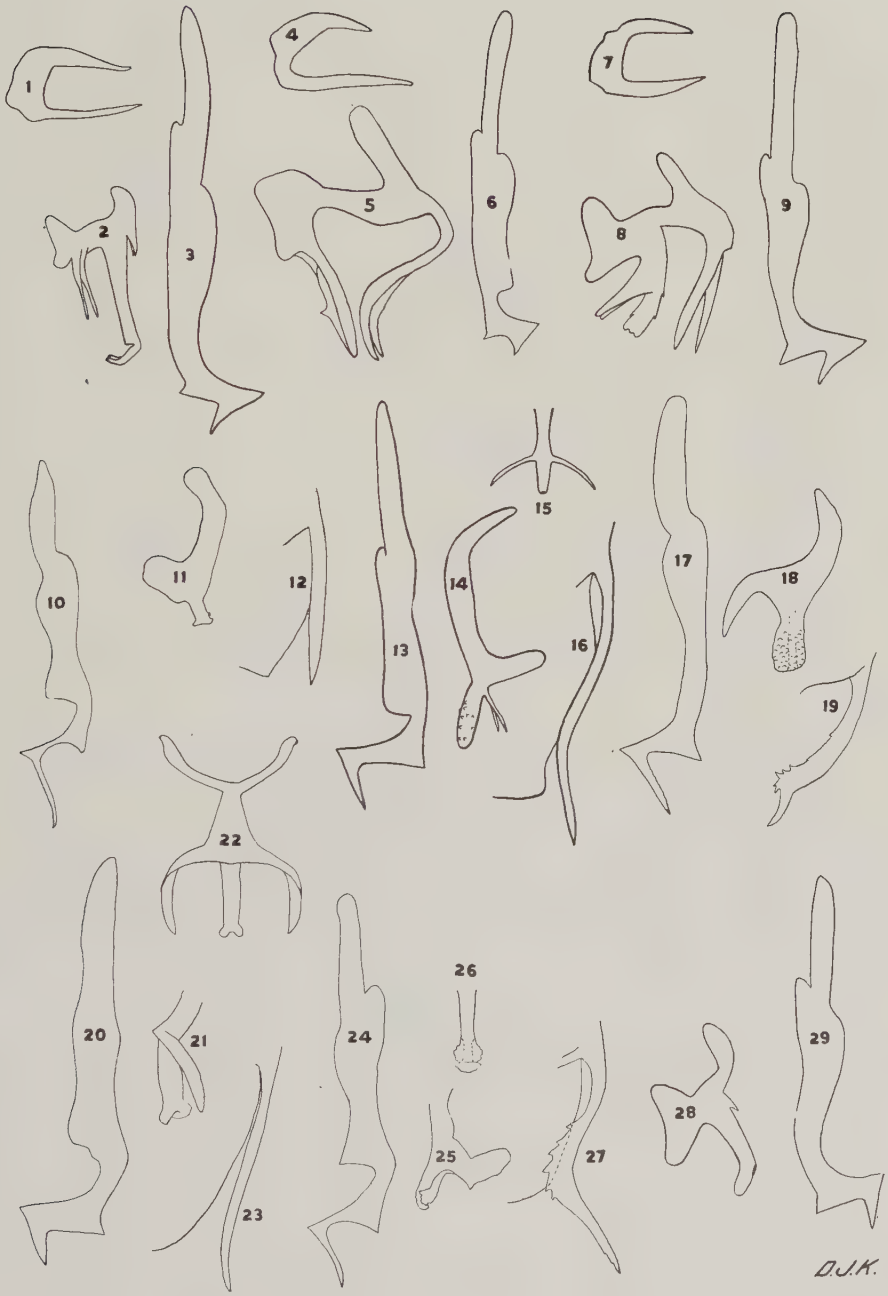
EXPLANATION OF PLATE I

Erythroneura kennedyi n. sp. Figs. 1, pygofer hook; 2, lateral view aedeagus; 3, style.
Erythroneura noncincta Johnson. Figs. 4, pygofer hook; 5, lateral view aedeagus; 6, style.
Erythroneura vagabunda n. sp. Figs. 7, pygofer hook; 8, lateral view aedeagus; 9, style.
Erythroneura corylorubra n. sp. Figs. 10, style; 11, lateral view aedeagus; 12, pygofer hook.
Erythroneura geronimoi n. sp. Figs. 13, style; 14, lateral view aedeagus; 15, ventral view aedeagus; 16, pygofer hook.
Erythroneura trautmanae n. sp. Figs. 17, style; 18, lateral view aedeagus; 19, pygofer hook.
Erythroneura paraesculi n. sp. Figs. 20, style; 21, lateral view aedeagus; 22, ventral view aedeagus; 23, pygofer hook.
Erythroneura stupkaorum n. sp. Figs. 24, style; 25, lateral view aedeagus; 26, ventral view aedeagus; 27, pygofer hook.
Erythroneura ohioensis n. sp. Figs. 28, lateral view aedeagus; 29, style.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apically orange-red markings. Vertex with three basal white spots, median longer, bordered by even band, pronotum with median Y-shaped mark, the arms reaching anterior margin, a large angular spot behind eye reaching halfway to posterior margin, scutellum with basal angles and apex orange; elytral markings diffuse and indistinct, in some specimens reddened on apical half. Clavi with basal anchor-shaped spot and one at apex; coria with indefinite area at base, spot opposite hyaline area of clavus and one at apex of clavus, plaque area irregularly colored. Crossveins and longitudinal veins brightly reddened, giving the elytral apices a reddish hue, small round black spot in base of cell M_4.

Inner male genitalia.—Pygofer hook single, point extending very slightly beyond pygofer, broad and parallel-sided to apical half where it is toothed on inner margin, turned sharply out near apex, then from turn narrowed abruptly to sharp spine-like apex. Style with rather small foot; heel large; base curved; anterior point projecting sharply laterad, posterior point longer than base of foot, straight, tapering to apex; aedeagus long, narrow and straight in ventral view; broad in lateral view.

Length: 2.9 mm.

Named for Dr. Mary Auten Trautman who collected the type series. Male holotype, July 17, allotype, July 18, and paratypes July 18 and 21, Hartwick St. Park, Mich.; paratype, Great Smoky Mt. National Park, Tenn., June 14, 1942, D. J. & J. N. Knull.

Erythroneura paraesculi n. sp.

A large brightly colored species of the maculata group near *E. aesculi* Beamer in external appearance.

Ground color creamy white on anterior parts and below, yellowish white on semihyaline elytra. Eyes gray, sometimes brown, markings of vertex, pronotum and scutellum pale orange and of usual maculata group type. A triangle of dark red occupies basal third of clavus based on claval vein and with apex at scutellar apex; another red vitta forms a heavy irregular U-shaped mark on elytra together, with its base including area just above apices of clavi and arms extending on coria not to costal margin; a small black dot anterior to costal plaque and outer crossvein reddened, an elongate black spot in base of inner apical cell. Elytra markings may be faint in specimens taken in June.

Male genitalia.—The unusual form of aedeagus readily separates this species. Style, posterior point almost half as long as base, sharply pointed and curved in; anterior point sharp, less than a right angle, slightly projecting; heel sharp, scarcely projecting, base thick. Pygofer hook long, slender, straight, tapering to fine point exceeding pygofer by one-sixth its length, curved gently ventrad. Aedeagus in lateral view with broad shaft bulging at middle, a ventral spine at middle and apex roughened with ventral and dorsal projections, heavy basal lateral processes as long as shaft extend laterad and ventro-caudad. In ventral view shaft narrow, broadened at apex where it is roughened and has lateral wing-like projections; heavy lateral processes extend straight out from base for half their length, then, broadened a little, extend down and forward, the whole forming a large m-shaped figure.

Female.—Last ventral segment long with median third well produced in a narrow lobe.

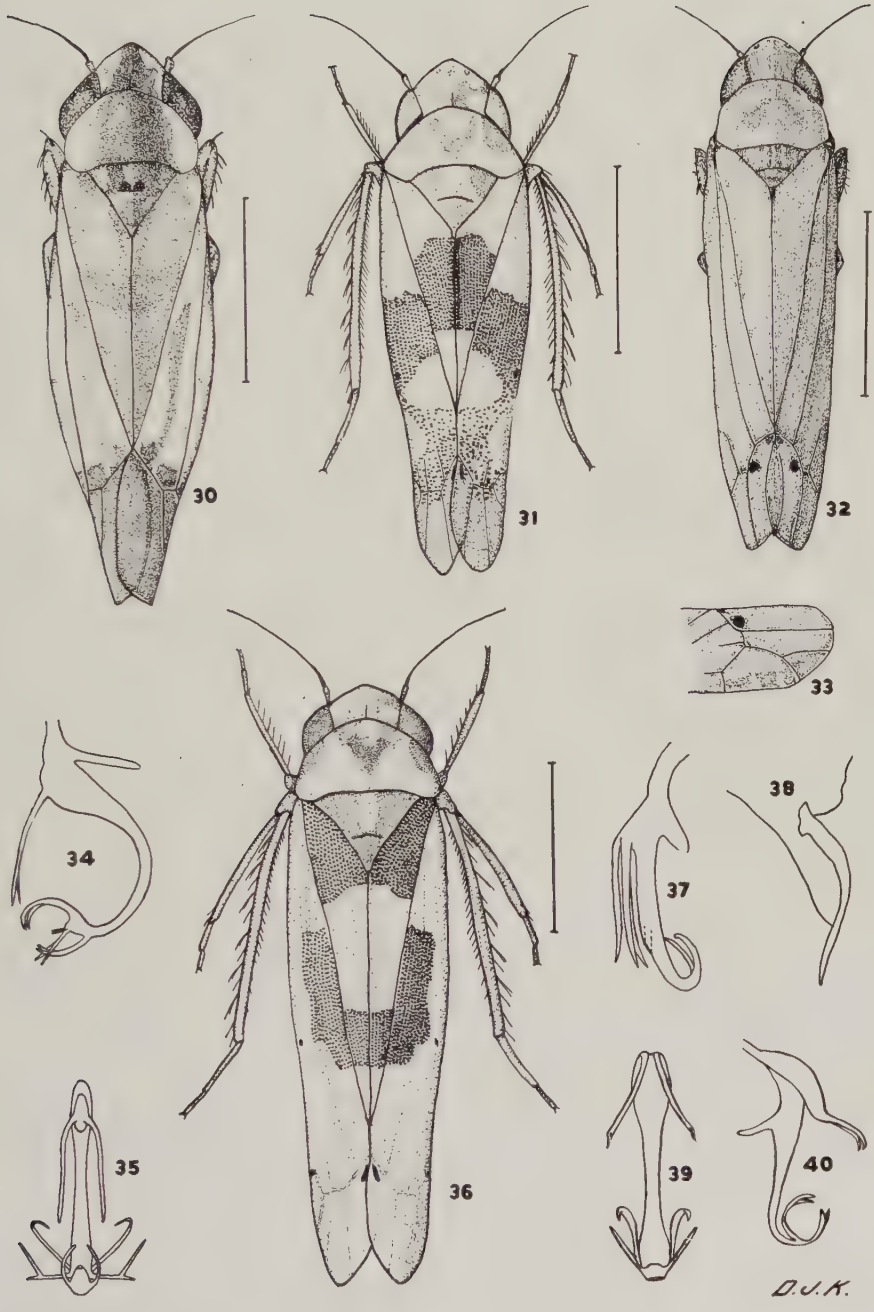
Length: 3.5 mm.

Male holotype, allotype and paratypes, Indian Gap, Smoky Mt. National Park, Tenn., June, 1942, D. J. & J. N. Knull, from *Aesculus*, and paratypes same locality, September 3, 1939, Mary Auten. Six paratypes in collection of The Ohio State University.

EXPLANATION OF PLATE II

(Line=1 mm.)

Fig. 30, *Erythroneura ohioensis* n. sp.
Fig. 31, *Erythroneura corylorubra* n. sp.
Alconeura socorroana n. sp. Figs. 32, dorsal view; 33, tip of elytron.
Typhlocyba sciotoensis n. sp. Figs. 34, lateral view aedeagus; 35, ventral view aedeagus.
Fig. 36, *Erythroneura paraesculi* n. sp.
Alconeura socorroana n. sp. Figs. 37, lateral view aedeagus; 38, pygofer hook.
Typhlocyba foliosa n. sp. Figs. 39, ventral view aedeagus; 40, lateral view aedeagus.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Erythroneura corylorubra n. sp.

This rare, minute, brightly ornamented species has been taken on *Corylus*, and belongs in the maculata group.

Head roundly produced one-half its length beyond eyes, eyes black, markings of head, pronotum and scutellum typical for this group, faint, yellow translucent and irregular (in one specimen collected in April these are distinct and orange); on each elytron an oblique vermillion vitta starts on clavus at apex of scutellum and covers a little more than median third, is continued across elytron from just above middle of claval vein to outer margin where it is broadened, a tiny black spot in lower posterior corner of vitta and a large one in base of inner apical cell. Crossveins and adjacent longitudinal veins both anteriorly and posteriorly broadly reddened giving appearance of vitta across apical area, apices yellowish semihyaline. Above white, semihyaline, shining. Below creamy white, apex of beak red, tarsal claws black.

Male genitalia.—Style with large slender foot, posterior point longer than basal width of foot and curved out at apex, serrate on inner apical two-thirds; anterior point projecting laterad, less than a right angle; heel prominent, rounded; base evenly curved, forming semicircle with basal half of posterior point. Aedeagus short and straight, in ventral view square-shafted with heavy curved lateral projections from apex, in lateral view with small dorsal tooth before apex and prominent lateral apical processes projecting dorsad; pygofer process heavy, almost straight, turned slightly ventrad, apex slightly exceeding pygofer, pointed, inner margin serrate.

Length: 2.5 mm.

Described from specimens from Hocking Co., Ohio, collected by D. J. & J. N. Knull. Male holotype, April 17, 1938; paratype, June 1, 1938; June 28; allotype and paratypes, August 31, 1944; September 14, 1944, and September 16, 1943.

Erythroneura geronimoi n. sp.

In maculata group near *E. biramosa* Beamer and *E. bigemina* McA., but with different head and pronotal markings; aedeagus with spines, and style differently formed.

White ground color with bright orange markings as follows: vertex with short transverse bar before apex, a pair of median basal prongs extending just above middle; eyes pale; pronotum with short blunt median Y on disc, triangular vitta behind either eye; scutellum with basal angles yellow, narrowly outlined with red, apex orange. Elytra: clavi with narrow basal hook-shaped mark and apical fourth; coria with small rounded spot on costa before humeral angle, angulate vitta surrounding costal plaque, becoming enlarged to somewhat diffuse before red crossveins; a large black spot at posterior end of plaque and a large one in base of cell M_4. Apical cells fumose. Below orange markings above antennal sockets, remainder creamy white.

Male genitalia.—Style with broad base, heel about a right angle, anterior point less than right angle, posterior point more than half as long as base of foot, narrow, sharp, meeting base at less than right angle curve. Pygofer hook long and slender, reaching beyond pygofer, curved gently in from base, then out on apical half. Aedeagus of medium length, straight in lateral and ventral view, a pair of lateral narrow spines arises at base behind aedeagus and extends laterodorsad. These spines vary in length but are usually evident. Those of the holotype which are figured are more pronounced than in most specimens.

Length: 3.25 mm.

A large series taken from oak, Chiricahua Mts., Arizona, by D. J. & J. N. Knull. Male holotype, allotype and paratypes, September 14, 1938, and additional paratypes, July 26, 1937. Ten paratypes in collection of The Ohio State University.

Named for Geronimo, chief of the Chiricahua band of Apaches.

Erythroneura ohioensis n. sp.

In the obliqua group, resembling *E. rufostigmosa* Beamer and *E. juncea* Beamer, but aedeagus with ventral basal spine and style with shorter, stouter posterior point.

Robust, pronotum one-third longer than vertex, venation of obliqua type. Eyes black, ground color creamy white with red dorsal stripe darkened interiorly, apical cells and spots before reddened crossveins fumose. On vertex, stripe, which extends over to face, projects laterally toward anterior corner of eye, and almost touches eye at base; gradually broadens on pronotum, somewhat irregular on sides; scutellum entirely dark; all of clavus except small pale humeral

area, orange red, darkened in scutellar area and inner middle, narrow orange red stripe on corium along inner vein on posterior two-thirds, stripe along costal margin from base to translucent orange plaque, broader and more opaque below plaque to crossveins. Below face reddish, lateral pale area above eyes in which is included a red prong above each antennal socket; cheeks pale, also venter and legs in female, in male abdomen darkened slightly ventrally, dorsum of abdomen with anterior segments black, showing through elytra.

Male genitalia.—Aedeagus from lateral view short, slender, gently curved dorsad, a short sharp baso-ventral prong; from ventral view straight, narrow, sharply pointed apex; style with medium foot, heel sharply projecting, base broad, anterior point projecting slightly laterad, about a right angle, posterior point broad at base, about half as long as base of foot, sharp.

Female.—Last ventral segment five times as long as preceding at middle, narrowed abruptly on apical third to produced median rounded lobe; black-tipped ovipositor slightly exceeding pygofer.

Length: 3 mm.

Male holotype, allotype and paratypes, Delaware Co., Ohio, April 30, 1944, D. J. & J. N. Knull; other paratypes same locality on following dates: April 25, 1943; April 26, 1944; May 6, 1943; July 8, 1942; September 19, 1943, and October 3, 1943. Six paratypes in collection of The Ohio State University.

Erythroneura kennedyi n. sp.

A member of the comes group in which it is near to *E. vaga* Johnson in male genital characters and between *E. omaska* Robinson and *E. beameri* Robinson in external appearance.

General ground color white marked with red. Vertex with median pale longitudinal oval reaching almost to apex of head and lateral basal oval spots, half size of median, entirely red margined, eyes dark. Pronotum with median Y-shaped mark, base narrow and longer than arms, touching both margins, a long even mark behind each eye, almost reaching hind margin. Scutellum, basal angles yellow, narrowly red-margined, apex red with a transverse white bar above it and space between basal angles white. Elytra with irregular zigzag vittae from humerus to crossveins with lateral narrow oblique extension below base and at anterior margin of plaque, a black dash parallel to them at lower edge of plaque; apex of clavus red, crossveins and adjacent longitudinal veins carmine; apical cells distinctly infuscated a short distance below crossveins, M_4 paler on disc; black spot at base of M_4 scarcely distinguishable from infuscation; and small distinct round spot at apex of R_3. Below, oval area either side on apex of head pale, red-margined, face pink, especially on margins, and clypeus red, remainder yellowish white, mesosternum and abdomen somewhat darkened; in male lateral portions of valve darker, and apices of plates black; in female apex of ovipositor black.

Genitalia.—Pygofer hook U-shaped, outer process almost as long as inner and about twice as broad. Foot of style small, heel prominent, base curved, anterior point very large, constituting about one-third width of foot, posterior point as long, but much narrower. Aedeagus long and slender, bifid at apex, arms forming transverse curved bar from ventral view and each arm one-fourth length of shaft, shaft straight, a pair of heavy dorsal spines arises behind shaft and extends parallel with it about two-thirds its length; separated from shaft at base by twice width of shaft. In some specimens a pair of stout spines arises anterior to base of shaft, in others this appears only as a lump.

Length: 3 mm.

This interesting leafhopper is named for Professor C. H. Kennedy.

Male holotype, allotype and paratypes, Pickaway Co., Ohio, February 20 and March 31, 1934, J. S. Caldwell; paratypes, Richland Co., Ohio, October 6, M. Auten. Five paratypes in collection of The Ohio State University.

Erythroneura vagabunda n. sp.

In the comes group near *E. aza* Robinson in genital characters and near *E. delicata* var. *accepta* McA. externally.

Background yellowish white, with orange to red markings. Vertex with median oval pale area and smaller circular area either side, narrowly red-margined. Pronotum, median Y with short base, longer arms not reaching anterior margin, dark color beneath showing through on

disc, broad irregular mark behind each eye. Scutellar basal angles yellow, narrowly red-margined, tip red. Elytra semihyaline, markings narrow, irregular, broken. Clavus with usual anchor-shaped basal mark, a black spot at suture, and small spot filling apex; corium with oblique dash on costal margin near base, another arising at anterior end of costal plaque enlarging toward middle of claval suture and angling to base of cell M_4. An oblique black dash at posterior end of plaque, obscure black spot in base of cell M_4, a definite one in apex of R_3, and apical cells infuscated some distance below red crossveins. Longitudinal veins white below crossveins, reddened a short distance above crossveins. Below sordid yellowish white, dorsum of abdomen and mesonotum black. Edges of front, clypeus and area around bases of antennae reddened.

Male genitalia.—Aedeagus from ventral view three-pronged, the median or shaft prong rough apically; pair of stout ventral processes straight, as long as shaft and parallel to it; pygofer hook U-shaped, outer arm a little thicker than and almost as long as inner. Foot small, heel prominent, base straight, anterior point short, about size of heel; posterior point heavy and twice as long as anterior point, shorter than base and meeting it at less than right angle.

Distinguished particularly by black dorsum of abdomen showing through elytra, black mesonotum and black spot at middle of clavus at elytral suture.

Length: 3 mm.

Male holotype, Lawrence Co., Ohio, April 29, J. S. Caldwell; allotype, Scioto Co., Ohio, June 17, 1944, D. J. & J. N. Knull; and paratypes from Hocking Co., June 1, 1938, D. J. & J. N. Knull; Jackson, Ohio, April 1, 1934, J. S. Caldwell; Chesapeake, Ohio, April 29, 1934, J. S. Caldwell; and Decatur, Ga., May 5, 1934, M. Auten. Two paratypes in collection of The Ohio State University.

Erythroneura noncincta Johnson

E. tricincta var. *noncincta* Johnson, Ohio Jour. Sci. 34: 261, 1934.
E. calycula var. *noncincta* Beamer, Univ. Kans. Sci. Bull. 24: 271, 1936.

Dr. Beamer stated in the above reference: "This is a variety of doubtful validity. . . ." As a fairly large series has been collected since its description, and also since the inner male genitalia show distinct characters, this variety is raised to specific rank and perhaps the shadow of doubt concerning its validity may be erased.

Male genitalia.—Pygofer hook U-shaped, with outer arm very short, scarcely one-fifth length of arm and of equal thickness; foot of style medium, heel small, base evenly curved; anterior point broad, longer than width of foot at narrowest place; posterior point short and sharp; aedeagus on dorsoventral view broad; shovel-shaped, with short lateral spine projecting either side, one-half distance from base, ventral processes curved toward shaft, slightly exceeding it, enlarged on outer half to twice basal width and tapering to sharp apices.

A male from Hocking Co., Ohio, April 15, 1934, J. S. Caldwell, is made an allotype and placed in the collection of The Ohio State University.

Xestocephalus piceus Osborn

Ohio Biological Survey Bulletin 14, p. 244, 1932.

The male is robust, uniformly dark brown above with exception of paler head, hyaline dots at inner middle and apex of clavus, and center of inner apical cell, square brown spot at costal margin just above crossvein surrounded by semicircular hyaline area. Below face, thorax and legs but for darker hind tibiae, straw-colored, abdominal segments dark with pale margins, and hairs of plates pale.

Resembles *X. brunneus* V. D. but is darker in color, larger and more robust. Taken in association with females exactly like type.

Length: 3.1 mm.

Male allotype, Hocking Co., Ohio, August 31, 1944, D. J. & J. N. Knull, and two males, September 14, 1944, in collection of The Ohio State University.

Dr. E. D. Ball, Jour. Wash. Acad. Sciences 22: 19, 1932, made *Twiningia irrorata* (Osborn) a synonym of *T. pellucida* (Ball). Examination of the type shows *T. irrorata* (Osb.) to be distinct and readily separable from *T. pellucida* (Ball) in a number of characters.

Unless stated otherwise type material is in collection of author.

A DESCRIPTION OF CALINELLA OPHIODONTIS N. SP. (TREMATODA, MONOGENEA) FROM THE LING COD, OPHIODON ELONGATUS GIRARD

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During the summer of 1942 a large specimen of *Ophiodon elongatus* was taken at Friday Harbor, Washington, of which the oral cavity was found to contain great numbers of a copepod, apparently a species of *Lepeophtheirus*. Adherent to these, in turn, were numerous adults, young, and eggs of a small, monogenetic trematode. Examination of the worms demonstrated that they markedly resembled members of the genus *Calinella* Monticelli, 1910. In a number of characters, however, they differed from previously described species of this genus. Accordingly, the worm is here considered as a species hitherto unrecognized for which the name *Calinella ophiodontia* is proposed.

SPECIFIC DIAGNOSIS

Calinella ophiodontis n. sp.

Udonellid with the characters of the genus *Calinella*. Of relatively large size; adults averaging 1.5 mm. in length, 0.25 mm. in breadth, with a posterior sucker 0.2 mm. in diameter. The male reproductive system contains a large seminal vesicle which takes the form of a double, tandem pouch. The ovary is about two-thirds as large as the testis, and a seminal receptacle is present connecting with the oviduct. The egg is 0.15 x 0.07 mm. and is equipped with a polar filament several times its length which ends in an attachment disk about 0.08 mm. in diameter (Fig. 3).

Locality: Friday Harbor, Washington.

Host: *Ophidon elongatus* Girard.

Location in host: On copepods in the oral cavity.

Type specimen: U. S. N. M. Helminthological Collection No. 36903.

DESCRIPTION

Calinella ophiodontis (Fig. 1) is a small cylindrical worm with a ringed cuticle covering its surface. At the posterior end of the body is a large, flattened sucker into which open many unicellular glands massed in the posterior part of the body. On the ventral surface, near the posterior end of the pharynx and commonly slightly to the right of the midline is the genital pore. On the ventral aspect of the anterior tip lies the buccal opening (Fig. 2) surrounded by a number of papillae or processes and equipped with a pair of small suckers. It opens into a thin-walled, prolapsed, buccal cavity, about which a number of apparently glandular cells can be detected. The buccal cavity is followed by a large, muscular pharynx which is fully protrusable. No esophagus is present, the pharynx leading immediately to a saccular intestine which extends to the posterior sixth of the body, and is more or less reflected dorsally about the genitalia. A small, dorsal excretory vesicle has occasionally been observed to open through the dorsal body wall at about the level of the posterior part of the uterus; other portions of the excretory system have not been identified.

The testis (Fig. 1) is an oval structure lying just posterior to the midlevel of the body and averaging 0.24 mm. in length by 0.19 mm. in breadth. From its left, anterior, ventral margin a vas deferens arises. This crosses the ovary obliquely and proceeds toward the genital pore to the right of the midline of the body. Near the posterior end of the uterus it swells into a vesicle,

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narrows, then enlarges into a second vesicle, and finally resumes its original diameter before joining the terminal end of the uterus, just posterior to the genital pore. As sperm are concentrated in the two enlargements it seems probable that these, together, make up the seminal vesicle. The short length of vas deferens (ejaculatory duct) external to these vesicles is ensheathed by a cluster of cells which may function as a prostate gland.

The ovary (Fig. 1), lying immediately in front of the testis, is slightly smaller than the latter, averaging 0.15 mm. in length and 0.20 mm. in breadth. From the left, ventral, anterior surface an oviduct arises and almost at once becomes swollen into a pouch, commonly containing a single mature ovum. The duct narrows and crosses the left, ventral surface of the ovary to the posterior margin where it is joined by a small duct from the seminal receptacle which lies between the ovary and testis. It abruptly bends forward and continues along the left margin of the ovary, receiving a duct from the yolk reservoir which lies ventral to the left-hand side of the ovary. This reservoir is formed by fusion of a right and left posterior, and a left anterior, yolk duct collecting material from the vitellaria surrounding the intestine. Anterior to the ovary the oviduct takes a somewhat oblique course to the midline of the body and becomes a thick-walled vesicle closely enveloped by numerous Mehlis' glands. It is questionable whether this vesicle can properly be considered as a cotype since it is always smaller in diameter than the completed egg. Beyond this structure the oviduct continues as a thin-walled uterus divisible into three sections: a pouch containing the attachment organ, a coiled, narrow portion containing the polar filament, and a distal sac containing the body of the egg. These divisions are detectable even when no egg is present. The saccular portion of the uterus is directed toward the right-hand side of the body. It narrows sharply and joins the vas deferens just before terminating at the genital pore.

It has been impossible to determine anything about the structure of the nervous system.

DISCUSSION

Guberlet (1936) in discussing *Calinella myliobati* raises the question of whether this worm should be included in the genus *Calinella* citing chiefly the paucity of North American records of Udonellidae and the somewhat distinctive arrangement of the vitellaria in this species. Since the parasitic fauna of North America still remains so incompletely surveyed the absence of records of this group can scarcely be adequately weighed at present. It is, also, doubtful whether any weight should be given to the arrangement of vitellaria in arriving at a generic distinction since, as Manter has shown (1926), this character may be variable within a single species. There could be little reason, then, for subdivision of the genus *Calinella* on the basis of our present knowledge. Indeed, quite the opposite view is taken by Price (1938) who regards *Calinella* as a synonym for *Udonella*.

In size, in form of the vas deferens and related structures, and in size and form of the egg there is certainly enough difference between *Calinella myliobati* and

EXPLANATION OF PLATE

All stated magnifications are those at which the figures were drawn; reduction in reproduction is indicated by a 5 centimeter figure accompanying the plate.

FIG. 1. Ventral view of entire worm with the yolk reservoir figured as semi-transparent to show the underlying structures. × 150.

FIG. 2. Anterior end showing buccal organization. × 250.

FIG. 3. Egg, removed from surface of copepod to show expanded attachment organ. × 200.

LIST OF ABBREVIATIONS

M—Mehlis' glands
O—Ovary
OO—Ootype
P—Prostate glands
SR—Seminal receptacle
SV—Seminal vesicle
T—Testis
U—Uterus
VD—Vas deferens
VF—Vitelline follicle
YR—Yolk Reservoir

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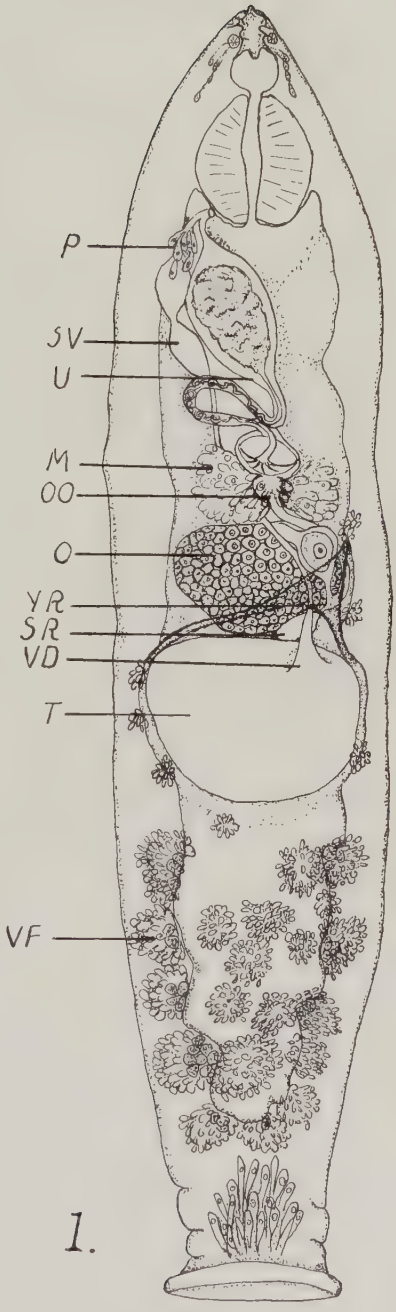

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Calinella craniola to warrant recognition of two species. The same is equally true of *Calinella ophiodontis:* it possesses fully the generic characters of *Calinella,* but it differs consistently and significantly from both *Calinella craniola* and *Calinella myliobati.* It may be distinguished from both species by the relatively large ovary and by the extremely long filament and the complex attachment organ of the egg. The form of the terminal part of the vas deferens is quite unlike that of *C. craniola* and differs somewhat from that of *C. myliobati.* The possession of glands opening into the posterior sucker has not been reported in this genus, though known in related forms. A seminal receptacle is not reported from *C. craniola,* incompletely described in *C. myliobati,* and definitely present in *C. ophiodontis.* Finally, the size of *C. ophiodontis* is considerably greater than that of *C. craniola* and slightly above that of *C. myliobati.* Taking these points into consideration it is scarcely possible to identify *Calinella ophiodontis* with either *Calinella craniola* or *Calinella myliobati.*

SUMMARY

Calinella ophiodontis n. sp. is described from *Ophiodon elongatus.* It is shown to differ from previously described species in size, in the possession of pedal glands, and in certain characters of the reproductive system and of the egg.

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REVIEW PAPERS

(Papers appearing in this section are invited reviews of subjects of wide current interest.)

A REVIEW OF THE PROBLEMS OF SULFONAMIDE CHEMOTHERAPY

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Every since the development of systematic bacteriology and the rapid growth of the germ theory of disease, man has been engaged in an untiring search for agents which would specifically destroy the offending organisms. Up to the time of Ehrlich, that search had been more or less fruitless. Ehrlich approached the problem from a fresh point of view, and it is to him that we owe our modern concept of the chemotherapy of disease. His theory was expressed as "therapia sterilisans magna." He chose to direct this "sterilizing therapy" at the dread scourge of syphilis, and in 1907 he announced the discovery of salvarsan. Ehrlich's was the first planned attack upon a specific organism by means of a specific drug. Up to that time, quinine was the only specific therapeutic agent known to medicine, and it had been used empirically. This date, 1907, then marks the beginning of the modern clinical attack upon disease-producing microörganisms.

In the next year, an obscure Australian chemist, Gelmo, synthesized p-aminobenzenesulfonamide. Chemotherapy was the farthest thing from his mind. He was searching for dye intermediates, and the astounding potentialities of his new compound remained unknown for more than twenty-five years. It was not until 1932 that two German workers, Meitzsch and Klarer, experimenting with the possibilities of various dyes as antiseptics, noted that Gelmo's compound was very effective in preventing death in mice infected with the β-hemolytic streptococcus. Little further work was done along the lines suggested by this discovery until 1935 when another German worker confirmed and extended the earlier observations and a new era in chemotherapy was opened. The compound with which he worked was not Gelmo's original p-aminobenzenesulfonamide but a derivative known as prontosil. It was shortly learned that the extra chemical group in prontosil was not necessary for its activity against the streptococcus and he named the parent group (Gelmo's original compound) sulfanilamide. The German scientist who thus opened the door to modern chemotherapy was named Domagk. In the same year three English workers, Buttles, Grey and Stephenson, confirmed these observations and made them known to the world. Domagk was awarded the Nobel Prize and it is a significant comment on trends then prominent in Germany that Hitler refused to permit Domagk to receive the award.

When we examine the structure of sulfanilamide, we see at once that a host of substitution products are possible from a strictly chemical point of view. It has been estimated that some 7,000 different compounds related to this nucleus have been prepared and tested biologically. Out of this huge amount of work has come a number of observations of fundamental importance with reference to the activity of the substitution products of sulfanilamide. Then it was learned that neither substitutions on the ring other than the p-amino group nor modifications of the sulfoxide radical would yield compounds which were effective against the bacteria. There remained then only the possibility of substituting the amide nitrogen. A large number of amido-substitution products have been prepared

but only a few of these have come into the practice of medicine. The first one of any importance was sulfapyridine, introduced in England in 1939. It was sylfapyridine which really established the value of this group of drugs in medicine, for sulfapyridine was effective against the pneumococcus, the most dramatic and one of the worst scourges of mankind. In 1940 American chemists gave us sulfathiazole, and in 1941 sulfadiazine appeared. Since 1941 there have been but few additions to the sulfonamide group of drugs. The substituted nucleus of sulfadiazine has been slightly modified and two of its relatives, sulfamethazine and sulfamerazine, are receiving the tests of their clinical value today.

It was further noted that substitution on the amino nitrogen made the compounds difficult to absorb from the gastrointestinal tract. Such compounds were found, however, to be effective against many types of bacteria which are either

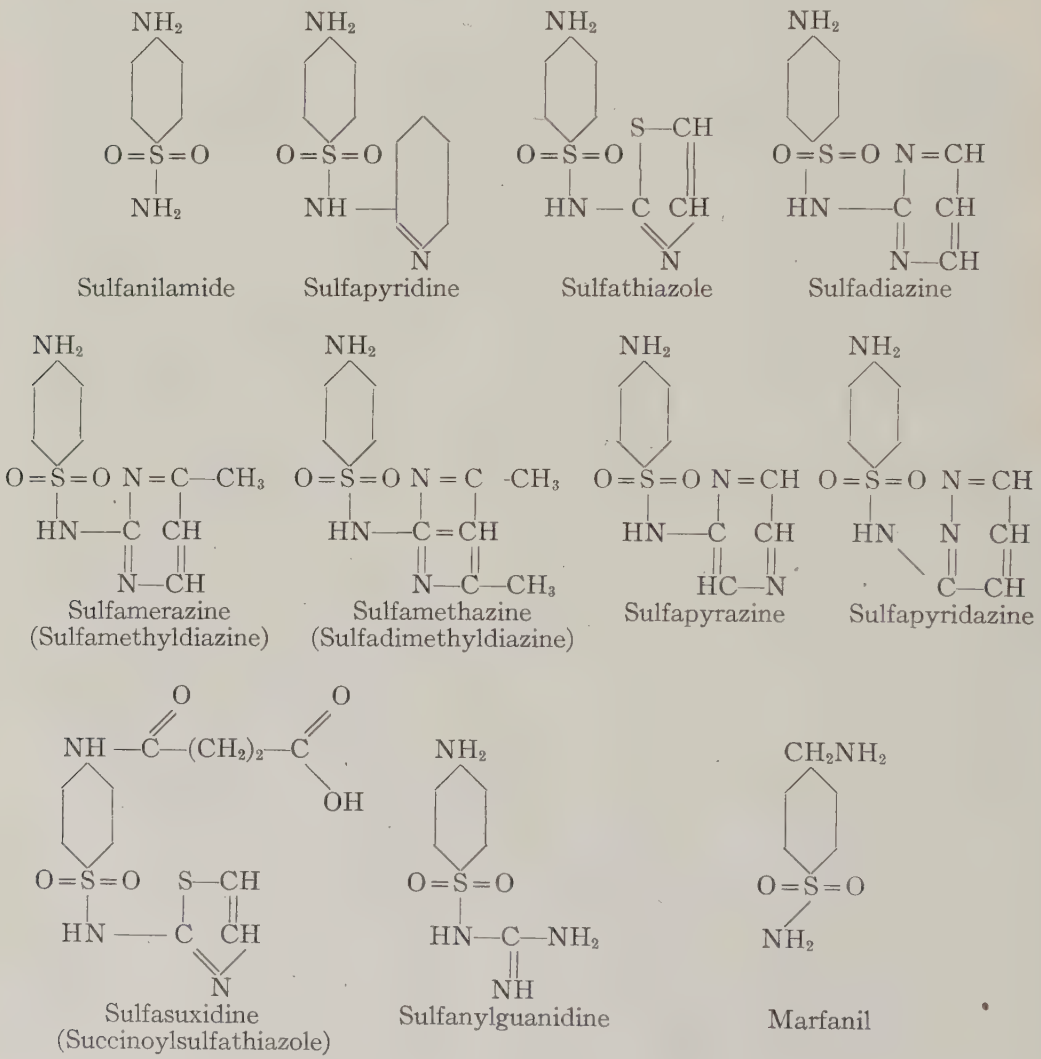

FIGURE 1. THE STRUCTURAL RELATIONSHIPS OF THE SULFONAMIDES

normal inhabitants of the gastrointestinal tract or produce diseases peculiar to that system. Two of these compounds have been of some use in medicine. They are sulfasuxidine and sulfanylguanidine. A third compound of this latter type known as marfanil has been employed by the Germans in the prevention and treatment of wound infections. Captured samples of this drug have been studied and its structure is known. Preliminary reports indicate that marfanil is more effective for the use noted than any of the previously known sulfonamides.

The structural relationships of all of these drugs are indicated in Figure 1.

PHARMACOLOGY

A. Absorption and Fate of the Sulfonamides.

All of the sulfonamides in which there is no modification of the amino nitrogen are relatively rapidly absorbed from the intestine. Maximum blood levels are attained in from 3 to 6 hours, following a single dose of any of the group. For this reason it is seldom necessary to administer the drugs by any other route than the oral. In certain specific cases, as, for example, when the patient is vomiting or in the presence of an overwhelming infection demanding heroic treatment, it is sometimes desirable to administer the drugs parenterally. With the exception of sulfanilimide, all of the free compounds are too insoluble for parenteral administration but for this purpose there are available soluble sodium salts of sulfapyridine, sulfathiazole and sulfadiazine.

All of the sulfonamides after absorption are rapidly distributed to all organs and tissues and to all of the extra-vascular fluid compartments. Concentrations attained are, in general, approximately 20 per cent lower than the concentration in the blood. One important exception to this general statement should be noted in the case of sulfathiazole. For some unknown reason, the concentration of sulfathiazole in the cerebrospinal fluid of the normal individual remains at a level represented by 10 per cent or less of the level in the blood. Recognition of this fact is important in the selection of a drug in the treatment of meningitis.

After the sulfonamides have been distributed throughout the body as noted above, very little happens to alter their chemical nature. All of them are acetylated by the liver to the extent of about 15 per cent of the total concentration in the blood. Recently it has been noted that certain members of the group tend to combine to a greater or lesser degree with the plasma. This action is spoken of as plasma-binding capacity. With sulfathiazole this occurs to the extent of about 25 per cent of the total plasma concentration; with sulfadiazine, to the extent of about 50 per cent; and with sulfamethazine, about 75 per cent. The variations in the plasma-binding capacity may be of significance with reference to the effective blood level in any given infection but the exact significance is not fully understood at the present time. Certain conditions existing in areas where infection is present or is likely to occur operate to lessen the effectiveness of the drugs. Among these must be mentioned collections of serum and the presence of pus. How these extraneous factors affect the metabolism of the sulfonamides is not known.

The chief route of excretion for all of the sulfonamides is via the urinary tract. Certain complications of great significance in the therapeutic use of the sulfonamides may occur as a result of this pathway of excretion. More detailed reference to this phenomenon will be made below.

B. Mechanism of Action.

The mechanism by which the sulfonamide drugs act to combat infectious processes presented a very difficult but interesting problem in bacteriology. Certain fundamental observations with reference to the classical theories of antibacterial action were soon made. They were as follows:

1. The drugs act by slowing the growth of the bacteria rather than by killing them outright; their action is bacteriostatic rather than bacteriocidal.
2. The drugs cause no potentiation nor inhibition of immunologic responses nor of the phagocytic mechanisms of the host.
3. The sulfonamides have no effect which could be interpreted as concerned with the neutralization of bacterial toxins.

In the light of these negative findings, it was necessary to postulate and study other possible mechanisms. The results of such studies may be briefly summarized as follows: The sulfonamides compete in the metabolism of the bacterium with some substance which is essential to the normal growth and multiplication of the organism. This hypothetic substance was definitely found to be p-aminobenzoic acid, known to the elect as PAB. Upon comparing the sulfonamides and PAB, it might be said somewhat facetiously, that the poor bacteria, never having had a college course in organic chemistry, are unable to distinguish between p-aminobenzoic acid and p-aminobenzene sulfonamide, and in attempting to utilize the former starve to death in the process. It is probably unwise to treat such an important discovery so lightly for, if it is possible to confuse the bacteria in this respect, then it is equally possible that a little research may disclose other substances which are essential to the well-being of the organisms. Synthetic compounds resembling these essential metabolites may then be developed and offered as ineffective substitutes for the essential metabolites to the detriment of the bacteria. The possibilities inherent in such an idea are so far-reaching that they stagger the imagination. Suffice it to say that already a great deal of research has been done along the lines suggested here. It is indeed fortunate that such a fundamental discovery has been made for it has been suggested only recently that the sulfonamides have probably been developed to their maximum extent. On the basis of certain careful physico-chemical observations, it has been predicted that sulfadiazine exhibits the maximum antibacterial action which can be developed by any derivative of the parent sulfonamide nucleus.

CLINICAL APPLICATION

A. Organisms Affected:

Following the initial wave of enthusiasm which greeted the introduction of the sulfonamides into the practice of medicine, it became quickly apparent that these new compounds were not panaceas. It was shortly learned that the sulfonamides were effective against a definitely limited number of organisms and that all of them were not equally effective against even these. Among the diseases which are most adequately controlled by the sulfonamides are those which are caused by the β-hemolytic streptococcus, the pneumococcus and the gonococcus. Among those diseases which may be fairly satisfactorily treated by the sulfonamides are those caused by the meningococcus, the staphylococcus, and the α-hemolytic streptococcus (viridans). It is thus apparent that the field of usefulness is limited almost entirely to the coccal infections. Infections in which the etiologic agent is a bacillus and which respond to the administration of the sulfonamides are uncommon. Chief among them are infections produced by *Escherichia coli*, and by the dysentery bacilli. The gas gangrene group of bacilli are only moderately susceptible. It must be emphasized that the sulfonamide drugs are totally ineffective against the causative agent of rheumatic fever and against the organisms causing typhoid fever, typhus, diphtheria, tuberculosis and syphilis. In addition to these, furthermore, none of the known virus infections responds to sulfonamide therapy. These include influenza, "virus" pneumonia, measles, smallpox and many others. Table I compares their relative effectiveness against the common organisms.

B. Special Cases:

1. Streptococcal Infections.

a. β-Hemolytic Streptococcus.—This organism is responsible for so-called strep throat; for one of the dreaded forms of blood poisoning; and very probably for scarlet fever. Once it has taken hold in a susceptible individual it goes like wildfire and the patient when first seen is usually acutely and dangerously ill. Complication follows complication and, if the patient is able to withstand the onslaught and survive, he must usually face a long period of debility.

b. α-Hemolytic Streptococcus.—This organism commonly known as *Streptococcus viridans*, or the "green-producing" streptococcus, is a common cause of upper respiratory infections of the type usually associated with the common cold and its sequellae. We have already noted that the sulfonamides are of limited value in the management of infections caused by this organism, and the use of these drugs in the common cold is generally of little value.

TABLE I

The Comparative Susceptibility of the Common Organisms to the Various Sulfonamides

Organism	Sulfanilamide	Sulfapyradine	Sulfathiasole	Sulfadiazine
β-hemolytic Streptococcus	++++	+++	+++	++++
α-hemolytic Streptococcus	++	++	++	++
Diplococcus pneumoniae (pneumococcus)	±	++++	++++	++++
Neisseria intracellularis (meningococcus)	++	++	++	++
Neisseria gonorrhoeae (gonococcus)	++	++	+++	+++
Staphylococcus	±	++	++	+++
Escherichia coli (urinary tract)	+++	++	+++	+++

One of the most terrible diseases known to medicine (fortunately, a relatively uncommon disease) is subacute bacterial endocarditis. This is a slowly progressive disease which nearly always attacks only those whose hearts have been damaged by rheumatic fever. It has been, up to the present era, a uniformly fatal infection. As one famous pathologist puts it, "The heart is beating muffled marches to the grave." Great hopes were held for its treatment when the sulfonamides became available, but disappointment and despair have followed their use. In one outstanding paper discussing the treatment of 67 cases of this disease, in which all of the sulfonamides and all adjuvant forms of therapy were used, the concluding sentence reads, "All sixty-seven patients are dead."

2. Pneumococcal Infections.

Prior to the advent of sulfapyridine, somewhere between 30 and 50 per cent of patients suffering from pneumonia died. Sulfapyridine alone reduced this appalling mortality to about 12 per cent and it was at once hailed as the "miracle drug." If no other benefits had resulted from the use of the sulfonamides, this victory alone would have been sufficient reward for the tremendous effort which has been expended in the development of these drugs. Sulfapyridine is no longer used in medicine, because both sulfathiazole and sulfadiazine are equally effective, and both of the latter are much less toxic to the patient than is sulfapyridine. In addition to the tremendous reduction in mortality it is apparent at once from this clinical picture that the morbidity has also been strikingly decreased. In a word, the fortunate patient who recovered from pneumonia in the pre-sulfonamide era was destined to spend long weeks of convalescence before he was able to return to anything resembling normal existence. Today he may be as "good as new" in as short a time as three weeks after the onset of the illness.

3. Gonococcal Infections.

Gonorrhea has always been held somewhat lightly in the minds of men. This attitude has prevailed in spite of the fact that gonorrhea rendered thousands upon thousands of women incapable of child-bearing. The misery, both physical and mental—and spiritual—produced by the gonococcus must forever remain incalculable. The sulfonamides have gone far in reducing this scourge, but the very nature of the disease and the circumstances surrounding its transmission work continuously against any method of treatment. We shall have occasion under another heading to refer again to the gonococcus.

Prior to the availability of the sulfonamides, an uncomplicated case of gonorrhea which was given the best therapy available could be cured in a matter of weeks. When the sulfonamides are used in a similar case today, the cure is literally a matter of hours. Here again we see strikingly illustrated the tremendous importance of these drugs.

C. Prophylactic Use.

It was natural that drugs which were so effective in the cure of certain diseases should be tried for the purpose of preventing these diseases from developing. For a long period of time after the introduction of the sulfonamides, their prophylactic use belonged in the category of "armchair" therapeutics. No one had any real information as to their efficiency in the prevention of disease and such use aroused a small storm of protest from those who were already beginning to realize the complications which might arise from the development of fastness in the organism and sensitivity in the patients. These men argued that before prophylactic use was justifiable, it must first be demonstrated that a sufficiently high degree of morbidity could be prevented to justify the danger of the production of sensitivity in those receiving the drugs, and to justify the development of "carriers" of sulfonamide-fast organisms. The multitude of variables presented in the consideration of these two problems has thus far defied analysis. The war with its consequent crowding together of large groups of men under conditions which have long been realized to be ideal for the development and spread of epidemics, made the prophylactic use of the sulfonamides under certain circumstances mandatory. For example, an epidemic of scarlet fever began on Navy Pier in New York City. At once a large section of the personnel was given sulfadiazine in small daily doses. The case incidence of scarlet fever in the treated group promptly fell to zero. Another trial of prophylactic value was made in a Southern army camp. Sulfathiazole was administered to each man who "signed out" in the evening, and a similar dose was given when he returned to camp. In this camp, prior to the introduction of routine sulfathiazole administration, the incidence of gonorrhea was 171 cases per 1,000 men per year. Following the prophylactic use of sulfathiazole, the case incidence dropped to 8 per 1,000 men per year. Many more such instances could be cited. However, they all point toward the same conclusion. It is possible by the controlled prophylactic administration of a sulfonamide to reduce materially the incidence of infection by any bacterial agent which is ordinarily susceptible to the sulfanomides. Thus the fact of prophylaxis is established. It must be emphasized, however, that the two dangers inherent in sulfonamide prophylaxis have not been adequately evaluated.

D. Toxicity

1. General Incidence of Toxic Reactions.

In the early years of sulfonamide chemotherapy, it was difficult to evaluate the incidence of total toxic reactions to the administration of these drugs. However, with the passing years it has become possible to give what is believed to be

a reasonably correct estimation of reaction incidence. The over-all reactions may be summarized roughly as follows (excluding the "mild" group in Table II):

Sulfathiazole.....18 per cent
Sulfapyridine.....16 per cent
Sulfanilamide.....12 per cent
Sulfadiazine.....10 per cent

In certain clinics it has been possible by meticulous attention to all details of therapy to reduce the over-all incidence of toxic reactions to figures appreciably below those cited. Table II gives a detailed analysis of the reactions which have been reported, together with an estimate of their distribution among the various sulfonamides. In general, those labeled "mild" do not contraindicate the further administration of the drugs; the label "moderately severe" should give reason

TABLE II

THE PERCENTAGE DISTRIBUTION OF THE MORE COMMON REACTIONS TO THE SULFONAMIDES

	Sulfanilamide	Sulfapyradine	Sulfathiazole	Sulfadiazine
	%	%	%	%
MILD				
Nausea and vomiting	25–40	40–60	6	0.7
Dizziness	50–60	50–60	5	rare
Mild hemolytic anemia	3	rare	rare	rare
Cyanosis	90–100	50	rare	none
MODERATELY SEVERE				
Acidosis	2	rare	rare	rare
Fever	10	4	6	3
Rash	2	2	6	2.1
Hematuria (microscopic)	?	–	4	5
SEVERE				
Leukopenia	0.3	0.6	1.6	1.2
Agranulocytosis	0.1	0.3	rare	rare
Anuria or oliguria	none	0.3	0.7	1.5
Jaundice	1.8	0.6	rare	rare

for stopping the drug unless to do so would endanger the patient's life; the appearance of these reactions called "severe" makes the cessation of administration mandatory.

Sulfadiazine is the most important of the sulfonamides today and has for most infections supplanted all the rest.

It is unwise to try to draw many conclusions with reference to the significance of these toxic phenomena from the bare figures given here. The severity of a reaction varies directly with the seriousness of the disease process which instituted the administration of the drug in the first place. These tables and figures are included merely to permit a general concept of the extent and type of toxic effects which may be anticipated.

It should be pointed out that the actual numbers of individuals receiving the drugs are so great that some of the figures given in the tables fade into insignificance. Only those reactions in which there may be a large element of sensitivity involved, and those reactions referable to the kidney will be considered in any detail. The significance of the "sensitivity" group is discussed in the section dealing with the limitations of the sulfonamides.

Those reactions referable to the kidney are of sufficient importance to merit further comment. These reactions are of two types. The first type is entirely mechanical in character. As the sulfonamides are excreted, both in their free

and acetylated forms, they are concentrated in the kidney. As that concentration rises they tend to be precipitated, and crystalline compounds thus formed act to produce mechanical damage to the kidney tubules. This damage may range from slight irritation of the tubules with the appearance of a little blood in the urine, to the complete blocking of the tubules followed by the loss of the ability of the kidney to function. In addition to these mechanical effects, the sulfonamides (especially sulfadiazine) produce a chemical action on the walls of the kidney tubules. This cellular damage may also result in serious derangement of kidney function.

The greatest attention has been paid to these reactions and, if the proper precautions are taken, the incidence of serious kidney damage can be reduced almost to zero. The precautions are simple indeed and involve, first of all, careful observation of the patient's urinary output, which should be at least 1000 cc. daily. In the second place, some effort should be made to keep the urine alkaline by the administration of an alkaline salt, such as sodium bicarbonate. An alkaline urine helps to insure maximum solubility of the drug and reduces the danger of damage by crystal formation.

It should be mentioned that the "dizziness" noted in the tables may make it unsafe for the patient to carry our skilled tasks, such as driving a car or piloting an airplane. For this reason, in the Air Forces, any pilot receiving a sulfonamide is "grounded" until his treatment is finished.

E. The Limitations of Sulfonamide Therapy.

1. Organisms Not Affected.

In a previous section, the sulfonamide susceptibility of the various organisms which commonly cause disease was discussed. Little more need be said here, except to point out that the use of the sulfonamides in diseases caused by unsusceptible organisms is to be unhesitatingly condemned.

2. Sulfonamide "Fastness."

In the early days of the sulfonamide therapy of gonorrhea, it was not uncommon to see reports indicating an incidence of cure as high as 90 per cent. As time has passed, this figure has further decreased until today one is likely to be depressed by the fact that rates of cure are being reported as low as 30 per cent. These carefully controlled case-studies are supported by the passing observations of many physicians who have not studied the problem in detail. For example, one prominent urologist in a Midwestern city told me in 1941 that he had long since cured all of the gonorrhea which was curable by sulfonamide and by sulfapyridine, and that he was then working on sulfathiazole. He remarked that he hoped a new sulfonamide would soon make its appearance else he would have to resort to the old pre-sulfonamide methods. These observations with reference to the gonococcus raised the question of the development of resistance by the organisms to the action of these drugs. This problem of resistance has been studied in great detail using all of the organisms which are known to be originally susceptible to sulfonamides. As a result of such studies, it is now known that most of the susceptible organisms under the proper conditions may become completely resistant to the action of the drugs. Such resistant organisms then are said to be sulfonamide-fast.

The conditions for the development of fastness have not been completely established. It seems, however, that prolonged exposure to concentrations of sulfonamide somewhat lower than those used therapeutically, is extremely effective in producing strains of resistant organisms. It is clear this phenomenon of fastness may be especially significant, if one takes the long term view. It is at once apparent that we must consider the definite possibility of the ascendency of a whole new order of sulfonamide-resistant bacteria.

It has been shown that about 40 per cent of persons who recover from pneumonia serve as carriers of the infecting organisms for a long period of time. Many of these organisms have undoubtedly become resistant to sulfonamides during the treatment of the active disease and it has been shown that even now the incidence of sulfonamide-resistant pneumococcal pneumonia is increasing.

The illustrations afforded by the gonococcus and the pneumococcus serve as a grave warning with reference to the future of sulfonamide therapy. The possibility of the development of resistant organisms constitutes one of the chief contraindications to the indiscriminate use of the sulfonamide drugs.

3. The Problem of Sensitivity.

A number of the toxic reactions discussed above have been found to be the result of the development within the individual of special sensitivity to the sulfonamides. This sensitivity is related in some fashion to the general problem of allergy. The exact mechanism of the development of sensitivity is not well understood. It is, however, assumed that the sulfonamides react in some fashion with certain protein elements in the blood-stream, and antibodies for this new compound are then developed. The result of these complex changes is a repetition of the toxic response following immediately upon the administration of the next dose of the sulfonamide which caused the original reaction. Here again, as in the case of fastness, all the factors producing such sensitivity have not been fully determined. It is the consensus of opinion, however, that both the size of the dose and the duration of the administration are involved. Some of the reactions presumed to be sensitivity phenomena, are fever, skin rashes, agranulocytosis, and hemolytic jaundice. Some of the other reactions may also belong in this category but there is sufficient question as to their being so classified that we shall omit them from this discussion.

In the case of fever it has been noted that it is most likely to occur between the 5th and 7th days of administration. When a patient who has developed a fever receives a second course of sulfonamide drugs within the next year, he is very likely to develop a drug fever within the first 24 hours. Now the relation of duration of administration to the development of the sensitivity reaction may be pointed out. If a first course of sulfonamide therapy does not extend into the period when the fever would ordinarily be manifest, then no sensitivity would be developed so far as fever is concerned. This conclusion is not entirely valid for exceptions to it have frequently been noted. The second major factor (the size of the dose) is difficult to evaluate. The use of the sulfonamides in the prophylaxis of infectious diseases necessitates the administration of smaller doses over a long period of time. In such circumstances the incidence of reactions of all kinds is very low, generally in the neighborhood of 1 to 2 per cent. However, following the cessation of prophylactic course, it has been suggested that the incidence of these reactions believed to be sensitivity phenomena is much higher whenever it becomes necessary to give those patients full therapeutic doses of the drugs. This observation suggests that it is possible to develop sensitivity by continuous small doses without any overt toxic manifestations during such a prophylactic course. In summarizing, it may be said that there is little doubt that some of the toxic reactions to the sulfonamides are sensitivity phenomena. The exact significance of such phenomena with reference to the future of sulfonamide chemotherapy is not yet apparent. Nevertheless, the possibilities are sufficiently alarming to discourage the indiscriminate administration of the sulfonamides.

THE FUTURE OF SULFONAMIDE CHEMOTHERAPY

As time goes on and we recover somewhat from the effects of our enthusiasm with respect to the sulfonamides, their value in the practice of medicine becomes more clearly defined. This group of drugs will continue to hold an important

place in therapeutics. The indications for their use have been clearly defined but the contraindications are still somewhat debatable. It is with the latter that we must be more concerned in the future. Obviously, the use of sulfonamides in the treatment of an infection caused by an organism which is known not to be susceptible to the sulfonamides constitutes a contraindication. Among the other contraindications, may be mentioned inadequate therapy, indiscriminate prophylactic use, and administration prior to an attempt to discover the nature of the offending organism. These three errors of application invite the development of "fast strains" of organisms. They also favor the development of sensitivity phenomena in the patients receiving them. If we are to keep the development of sensitivity and the development of fastness at a minimum, then it behooves us to use the sulfonamides sparingly and only after weighing carefully all of the dangerous possibilities which may, and inevitably will, accrue from their indiscriminate use.

It is probably justifiable to say that the sulfonamides themselves have served their greatest purpose in the treatment of human ills, not by virtue of their effect upon certain specific organisms, but because they have introduced us to a new approach to the problem of bacterial infection in general—the so-called metabolic theory of bacteriostasis. There is every reason to believe that other substances essential to the welfare of a given organism will be discovered. Then compounds may be prepared which will compete with that essential metabolite to the detriment of the organism in question. From this point of view, the outlook for the future of chemotherapy is indeed bright and it is not inconceivable that some time in the near future we may hope for drugs which will rid us of all infectious diseases.

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